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QUARTERLY

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IN THIS ISSUE

NUTRITIONAL deficiencies in their preclinical states have been found to be very common among all economic groups of our population. The circumstances producing a situation of such importance to national health and vitality are discussed by Dr. Norman Jolliffe in an article entitled, "Nutritional Failures: Their Causes and Prevention." Dr. Jolliffe points out that the causes are manifold and include such varied causes as food processing methods, individual food habits, knowledge of food values, poverty, and the effect of illness on nutritional status. The best efforts of professional persons in many fields and intelligent cooperation of the public are required to solve the nutrition problem which is so fundamentally affected by cultural, social, and economic aspects of our national life. The important contribution which the practicing physician and the public health worker can make to a national program for better nutrition is discussed by Dr. Jolliffe, who offers many specific and practical suggestions.

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When the National Socialists came into power in 1933, Hitler not only launched into a vast construction and armament program; he deliberately set out to increase the birth rate through various schemes such as marriage loans and grants to large families. That the sharp rise of the birth rate following Hitler's accession to power was a real situation is conceded by impartial students. The question remains as to whether this abrupt reversal in the trend of the birth rate was due directly to the population policies instituted by Hitler or whether it was due to other socio-economic changes. In an article, "The Relation of Employment Levels to Births in Germany," Mr. Dudley Kirk adduces evidence that, in Germany and other countries, indices of marriages and births react sensitively to abrupt changes in employment conditions. The data

strongly suggest that a major part of the rise in German births after 1933 can be attributed to reemployment in the construction and armament program rather than to any specific National Socialist population policy.

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The significance of visual threshold in dim illumination as an index of nutritional status with respect to vitamin A has not been clearly demonstrated despite numerous investigations. Failure to improve the visual thresholds of subjects to whom vitamin A was administered over a period of six weeks is reported by Eleanor P. Hunt and Kenneth M. Hayden in "Variability of Visual Threshold," which is the ninth in a series of reports on Medical Evaluation of Nutritional Status. The authors also present a detailed statistical analysis of the extent of individual variations in threshold which are to be expected as the result of errors of measurement, and as the result of temporal change in an individual's threshold.

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The high prevalence of dental defects among young men examined for military service has brought this health problem into prominence during the past year. Data from a survey among high school students in New York City are the basis for an interesting epidemiological observation concerning the comparative frequency of dental conditions among children from different socio-economic groups. The numbers of teeth attacked by dental caries, past and present, per child examined were found to be equal among high school students from families of high income and from families of low income. Dr. Henry Klein discusses these findings in the tenth report in the series on Medical Evaluation of Nutritional Status, "Susceptibility to Dental Caries and Family Income." The author concludes that "caries susceptibility is determined by factors other than environmental variables associated with socio-economic status." Although dental care prevented extractions among children of high-economic status, it had not prevented the occurrence of caries to an extent equal to that among children from low-income families.

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Plasma content of ascorbic acid has been used increasingly in the past few years as a diagnostic criterion for the subclinical state of ascorbic acid deficiency. Determination of the amount of ascorbic acid in plasma is

made easily and objectively by use of the photoelectric colorimeter, and this method has proven valuable for surveys and other types of investigations. In the paper, "An Analysis of Sources of Errors in the Photelometric Macromethod of Determining Ascorbic Acid in Plasma," one of a series on Medical Evaluation of Nutritional Status, Miss Dorothy G. Wiehl and Myron Kantorovitz discuss the accuracy of findings on plasma specimens which may be expected under specific conditions. When important sources of potentially large errors are controlled, ascorbic acid content is determined with a high degree of accuracy.

NUTRITIONAL FAILURES: THEIR CAUSES AND PREVENTION[1]

NORMAN JOLLIFFE, M.D.

NUTRITIONAL failure[2] begins when adequate amounts of essential nutrients are not provided to the "milieu interne." From this point the successive stages are represented by tissue depletion, biochemical "lesions," impaired function, and finally by anatomical lesions.

The anatomical lesions which occur in such clinical states as hunger edema, rickets, osteomalacia, scurvy, beriberi, and pellagra have long been recognized. There are a number of anatomical lesions, however, which are only beginning to be recognized as due to nutritional failure. Among these are cheilosis, fissures in the angles of the mouth, filiform dermatosis in the nasolabial folds, and invasion of the cornea by capillary blood vessels, all commonly attributed to nutritional failure related to deficiency of riboflavin. Quite recently Kruse (1) pointed out that xerosis conjunctivæ is probably the earliest anatomical lesion of nutritional failure related to deficiency of vitamin A.

Evidence has accumulated during the past five years which indicates that the diagnosis of nutritional failure cannot be limited to clinically manifest anatomical lesions. The preclinical states, as represented by tissue depletion, biochemical "lesions," and altered physiology, hold greater import because they precede and are more common than the anatomical lesions. These preanatomic lesions cause inefficiency, impair well-being, and constitute potent sources of poor health.

[1] This paper is taken from the chapter, "Nutrition and the Deficiency Diseases" by Dr. Jolliffe in the book PREVENTIVE MEDICINE IN MODERN PRACTICE, edited by the Committee on Public Health Relations of The New York Academy of Medicine, to be published shortly by Paul B. Hoeber, Inc., 49 East 33rd Street, New York.

[2] For the purpose of this discussion, nutritional failure is limited to deficiencies of protein, minerals, and vitamins.

Causes Contributing to Nutritional Failure

The circumstances mainly responsible for nutritional failure in this country can be classified under four headings: Poor food habits, highly-milled grain and refined sugar, price rationing, and intercurrent illness. None of these factors stands alone but each is inextricably interwoven with the others.

Poor Food Habits. Poor food habits develop in part out of the economic factors which are expressed by the term "price rationing"; they also foster and are a part of the wide use of highly-milled grain products and refined sugar. While ignorance of the rules of good nutrition is fundamental in the causation of poor food habits, they are dependent in considerable degree on popular taste and social custom, factors which weigh heavily in accounting for the consumption of significant quantities of nutritionally inferior foods.

Poor food habits may be either negative or positive. The negative poor food habits include nonconsumption of adequate amounts of the protective foods. This is often due to failure to promote the taste for the protective foods during childhood, to local food customs, racial antipathies, and economic restrictions. In many instances it is due to faulty social conditioning, as when the adolescent boy stops drinking milk as soon as he dons long trousers. The positive poor food habits include excessive use of candy, sweet carbonated beverages, and alcohol. The amount of candy sold in this country in 1939 was sufficient to furnish 90 calories per capita per day. The amount of alcohol consumed in the United States in 1938 was sufficient to furnish about 86 calories per capita per day, and probably exceeded 200 calories per person of alcohol-consuming age (2). Data on the amount of calories furnished by sweetened carbonated beverages to our thirsty population are not available at the present time. It must be very considerable, however, and it may exceed the calories furnished per capita by alcohol and candy combined. It is especially pertinent to note that, except for the small portion which is consumed in the home, these three sources of calories were not taken

into consideration in the Bureau of Home Economics surveys (3). In the case of alcoholic beverages even the amount consumed at home was not counted (4). Yet these vitamin-free calories, constituting possibly from 10 to 15 per cent of the total caloric intake, play an important part by lowering the vitamin-calorie ratio in depressing the quality of our dietary. This fact can be illustrated by adding 250 vitamin-free calories to Sherman's average American diet, which contains 2,500 calories and 342 International units of thiamin and has been calculated (5) to provide a 20 per cent margin of safety in vitamin B_1 above the level where clinical signs of deficiency may appear. With 250 vitamin-free calories added, the safety margin is reduced to only 10 per cent. Since this is an average diet, a 20 per cent safety margin means that considerable numbers have little or no margin of safety. This is actually the case when the safety margins of the city diets studied by Stiebeling and Phipard (3) are calculated (5). The effect of reducing this tenuous figure by half can be readily appreciated.

Vitamin-Free or Poor Calories. The factor of highly-milled grain products and refined sugar (white flour; white bread; highly-milled cereals; granulated, cane, and beet sugar) has contributed enormously to the present poor nutritional milieu in our country. It has already been indicated how this is bound up with food habits, white bread and sugar being widely considered as social refinements, besides appealing to popular taste. Economic necessity, however, is also involved in promoting the use of these products, for they are the cheapest sources of calories.

As pointed out by R. M. Wilder (6), undernutrition is now more serious than at any time in our history. It is worse today mainly because detrimental changes in our dietary habits which started sixty years ago have progressed almost uninterruptedly up to the present time, so that there is a cumulative as well as a more inclusive effect.

Some real improvements have occurred in the American dietary since 1880, notably the greater and more widespread distribution of

fresh vegetables, milk, and meats. This began with the development of the railroads, permitting stable foods indigenous to one section of the country to be distributed in other sections. Then came the railroad refrigeration car and express freight, which made possible the rapid transportation and hence wide distribution of perishable foods, such as meat, fresh vegetables, and milk. Next, the development of the canning industry has resulted in the preservation of fruits, vegetables, and meats at the season of the year when they are most abundant and cheapest, and their shipment and distribution all over the country throughout the year. A final factor in the improvement of our dietary has been the development of the motor truck. While commercial fresh fruits and vegetables were at one time available only in the fairly good-sized towns and cities, there is now hardly a hamlet in our entire country that cannot have these foods delivered to it by motor truck.

Other far-reaching changes in the American dietary, however, which have been called "improvements" have actually been detrimental. The first of these is the patent milling process. Prior to 1880 our grain was milled almost entirely in local communities, by methods which retained most of the vitamins and minerals. Since the introduction of the patent process from Hungary by way of England, grain has come to be milled more and more in the region around Minneapolis, and then transported to all sections of the country. The patent milling process, by removing most of the germ and the bran shorts from the flour, renders it much less susceptible to spoilage. This feature recommended the patent process to the millers and food distributors. The American people, at the same time, preferred the resulting white, cleaner, more uniform, and better baking flour. This process has caused the removal of about 90 per cent of the vitamin B_1 from flour, and 70 to 85 per cent of other fractions of the vitamin B-complex, and of iron.

The second major "improvement" in the American diet which has had a detrimental effect has been a marked increase in the con-

sumption of sugar. About one hundred years ago our annual per capita consumption of sugar was not more than ten pounds. Since the Civil War, and especially since 1910, this has progressively increased so that by 1939 our consumption of sugar amounted to more than one hundred pounds per capita per year. These two sources of calories, white flour and refined sugar, make up about 55 per cent of the calories consumed by the American people, and this total source of calories does not provide more than 50 International units of vitamin B_1. Thus, "a 55 per cent fraction of the calories in the American diet of 1840 containing a minimum of 600 I.U. of vitamin B_1 has been replaced in the contemporaneous American diet by a like fraction containing only about 50 I.U." (5) and has been correspondingly reduced in the other B vitamins and iron. These two factors, white flour and white sugar, must bear much of the blame for the present nutritional inadequacies in our country. The reduction of vitamins and minerals in our dietary brought about by the substitution of white flour for whole wheat is illustrated in Table 1. It will be noted that only 11 per cent of the thiamin originally present in whole wheat survives in flour made by the patent milling process, whereas stone grinding preserved nearly 80 per cent of this vitamin, and proportionate amounts of the other nutritive essen-

Table 1. Vitamin and mineral content of flour.

	Whole Wheat[1]	White Flour[1]	Reduction	Enriched Flour[2]	
			Per Cent	Min.	Max.
Thiamin (mg. per lb.)	2.04	0.23	89	1.66	2.5
Riboflavin[3] (mg. per lb.)	1.13	0.18	85	1.2	1.8
Nicotinic Acid (mg. per lb.)	12.3	3.7	70	6.0	9.0
Iron (mg. per lb.)	18	4.5	75	6.0	24
Calcium[4] (mg. per lb.)	240	72	70	500	2,000
Phosphorus (mg. per lb.)	1,700	460	73	—	—
Vitamin D[4] (I.U.)	—	—	—	250	1,000

[1] Council on Foods and Nutrition, American Medical Association (7).
[2] Food and Nutrition Board, National Research Council; and U. S. Food and Drug Administration (8).
[3] Inclusion in "enriched" flour and bread will not be required, but is optional until July 1, 1942.
[4] Inclusion in "enriched" flour and bread is optional.

tials. The effect of widespread use of patent flour, plus the tenfold rise in consumption of sugar, has been offset only slightly by increased consumption of vegetables, fruits, and dairy products; very slightly, if at all, in the low-income groups who do not purchase these relatively more expensive sources of calories. It has been calculated (5) that it would require the daily consumption of 625 gm. of fruit, 600 gm. of potatoes, 880 gm. of other vegetables, and 1,260 cc. of milk to make up the difference—a combination impossible because of its bulk.

Price Rationing. The third factor responsible for our nutritional depression, price rationing, is one which constantly confronts all those interested in public health. Stated in simple terms it means that about half the people in our country have available for expenditure on food only three-fourths the minimum cost of a nutritionally satisfactory diet. Like the other factors, price rationing does not stand alone but is a part of and a cause of poor food habits, poor budgeting of available resources, and the established use of the highly-milled grain products and refined sugar. Moreover, it is set within a framework of individual, group, and national resources and requirements, and of industrial, social, and economic policies. For this reason, although this aspect of the national nutrition problem belongs properly in the realm of economics, the physician and public health worker must take it into account in planning a nutritional program.

Intercurrent Illness. The fourth factor responsible for nutritional failure is the neglect to prevent and correct malnutrition during and after illness. Methods for the prevention and treatment of specific nutritional failures during illness and convalescence will be given in the following section.

A Program for Better Nutrition

A program for better nutrition consists logically of two main parts. The first is the prevention and eradication of nutritional fail-

ure due to intercurrent illness. In this program the practicing physician plays the chief rôle. The second part consists of a public health nutrition program for which the public health authorities must be chiefly responsible.

Prevention and Eradication of Nutritional Failure Due to Intercurrent Illness. Nutritive impairment may be associated with almost any disease, and in many instances is increased by customary therapeutic procedures. The rôle played by these two factors, that is, intercurrent illness and therapy, in precipitating nutritional failure has scarcely been recognized. It is especially common in patients who have had poor food habits or belong to the groups subject to the price-rationing factor. It is here that the practicing physician can play a major rôle in a program for better nutrition. In Table 2 are listed some of the more important illnesses in which nutritional failure may occur.

The chief reliance for maintaining an adequate supply of all essential nutrients during any illness must be in food, for an adequate diet provides essential proteins and many vitamins impossible to encapsulate or provide in ampules or tablets. The foods around which a diet for the duration of illness and during convalescence should be built are those easily eaten, easily digestible, which provide relatively small residue, and at the same time provide as nearly as possible all the nutritive essentials. Such a diet, given in Table 3, is in use by the author, and seldom requires alteration.

This diet provides a safety margin over the allowances of the Food and Nutrition Committee of the National Research Council of about 100 per cent in riboflavin and ascorbic acid, and over 50 per cent in calcium and iron; it just meets the allowances in vitamin A and thiamin. It is probable that the allowance for nicotinic acid is considerably exceeded. The mere fact, however, that there is no safety margin for vitamin A, a fat-soluble vitamin, and for thiamin, an important member of the group of B vitamins, emphasizes the gross inadequacy of many diets provided to patients during illness.

I. *By Interfering with Food Intake*
- 1. Gastrointestinal Disease
  - Acute Gastroenteritis
  - Cholecystitis and Cholelithiasis
  - Peptic Ulcer
  - Diarrheal Diseases
  - Carcinoma of Stomach and Esophagus
- 2. Food Allergy
- 3. Mental Disorders as
  - Neurasthenia
  - Neurosis
  - Psychoneurosis
  - Psychosis
- 4. Operations and Anaesthesia
- 5. Infectious Diseases Associated with Anorexia
- 6. Loss of Teeth
- 7. Heart Failure (Anorexia, Nausea, and Vomiting by Visceral Congestion)
- 8. Pulmonary Disease (Anorexia, Vomiting Due to Cough)
- 9. Toxemia of Pregnancy (Nausea and Vomiting)
- 10. Visceral Pain (As in Renal Colic, and Angina that Reflexly Produces Nausea and Vomiting)
- 11. Neurologic Disorders Which Interfere with Self-Feeding
- 12. Migraine

II. *By Interfering with Absorption*
- 1. Diarrheal Diseases as
  - Ulcerative and Mucous Colitis
  - Intestinal Parasites
  - Intestinal Tuberculosis
  - Sprue
- 2. Gastrointestinal Fistulae
- 3. Diseases of Liver and Gallbladder
- 4. Achlorhydria
- 5. Carcinoma of the Stomach

III. *By Interfering with Utilization*
- 1. Liver Disease
- 2. Diabetes Mellitus
- 3. Chronic Alcoholism

IV. *By Increasing Requirement*
- 1. Abnormal Activity, as Associated with
  - Prolonged Strenuous Physical Exertion
  - Delirium
  - Manic-Depressive Psychosis
- 2. Fever
- 3. Hyperthyroidism
- 4. Pregnancy and Lactation

V. *By Increasing Excretion*
- 1. Polyuria, as in
  - Diabetes Mellitus
  - Diabetes Insipidus
  - Long Continued Excessive Fluid Intake, as in Urinary Tract Infections
- 2. Lactation

Table 2. Illnesses that may contribute to nutritional failure.

This applies to a great number of diets given in text-books for use either as an adjunct or as the specific therapy of many diseases. The necessity of supplementing even a diet as adequate as this with either a daily portion of beef liver (not calf liver) or providing at least vitamin A and thiamin, if the illness is of more than a few days' duration, is thus obvious.

By appropriate substitutions this diet can be made to suit almost any patient and illness. If the patient is stuporous and requires tube feeding the following changes should be made: The meat, butter,

(a) *Food*	*Amount*
Orange Juice	500 cc. (16 oz.)
Milk (Vitamin D)	1,000 cc. (1 quart)
Cream (20 per cent)	200 cc. (7 oz.)
Butter	30 Gm. (1 oz.)
Eggs	2
Meat (lean), Fish, Chicken, or Liver	200 Gm. (7 oz.)
Cereal (Whole Grain), Cooked (or Dry, 30 Gm. or 1 oz.)	200 Gm. (7 oz.)
Bread (Whole Wheat or "Enriched")	200 Gm. (7 oz.)
Sugar (or Honey, Jam, Marmalade)	30 Gm. (1 oz.)
(b) *Nutritional Essentials Furnished by Above Diet*	
Calories	2,500
Carbohydrate	210 Gm.
Protein	125 Gm.
Fat	135 Gm.
Calcium	1.6 Gm.
Iron	17 Mg.
Vitamin A	5,300 I.U.
Vitamin D	(Over) 400 I.U.
Thiamin	2 Mg.
Riboflavin	4.5 Mg.
Ascorbic Acid	250 Mg.

Table 3. Constituents of a diet for sick patients.

and bread are omitted. In their place eight egg whites, an additional 150 cc. of 20 per cent cream, and 60 gm. of barley flour made into a gruel, are given. These foods plus the milk, cream, and sugar can then be combined and water added to a volume of 2,500 cc., and given through a nasal catheter in twenty hourly feedings of 125 cc. The orange juice should be given separately in four feedings of 125 cc. For patients extremely ill, though not requiring tube feeding, the same mixture can be given by mouth at frequent intervals.

When a diet of less residue is required, skimmed milk may be substituted for whole milk, 200 gm. of cottage cheese may be substituted for the cream and butter, the bread should be white enriched, and the cereal, farina; the orange juice should be strained. When a high residue diet is required the orange juice should be replaced by whole orange, and leafy green and yellow vegetables should replace a part of the cereal or bread.

Table 4. Nutritive composition of more common foods.[1] Amounts per 100 grams.

	Calories Number	Carbohydrate Grams	Protein Grams	Fat Grams	Calcium Mg.	Phosphorus Mg.	Iron Mg.	Vitamin A I.U.*	Thiamin Mg.	Riboflavin Mg.	Nicotinic Acid Mg.	Ascorbic Acid Mg.
Dairy Products												
Cows Milk, Whole	69	5	3.5	4.0	120	93	0.24	110	0.05	0.20	0.08	Tr
Cows Milk, Whole, Dried	690	50	35.0	40.0	1,200	930	2.40	875	0.30	1.5	0.8	Tr
Cows Milk, Skimmed	36	5	3.4	0.3	122	96	0.25	2	0.05	0.20	0.09	Tr
Cows Milk, Skimmed, Dried	365	51	34.0	3.0	1,220	960	2.50	20	0.375	1.80	0.90	Tr
Cheese (Except Cottage)	455*	4.1*	27.0*	36.0*	930*	680*	1.4*	2,500	0.04	0.75	0.20	Tr
Cheese (Cottage)	110	4.3	21.0	1.0	82	263		500				0
Butter	770	0	1.0	85.0	Tr	Tr	0	2,500	Tr	0.3	Tr	Tr
Cream 20 Per Cent	200	4.5	2.5	18.5	86	67	Tr	600	Tr	Tr	Tr	Tr
Eggs (Whole)	150	0	13.5	10.5	67	180	2.50	1,000	0.15	0.5	0.3	0
Egg White	46	0.8	10.8	0	15	14	0.10	0	Tr	0.23	1.5	0
Bread												
Whole Wheat	245	50	9.7	0.9	50	175	1.6	0	0.42	0.08	1.8	0
Wheat White	260	53	9.2	1.3	27	93	0.9	0	0.04	0.03	0.7	0
Wheat White, Enriched	260	53	9.2	1.3	70	93	0.83	0	0.2	0.13	2.2	0
Flour												
Whole Wheat	355	72	13.8	1.9	50	350	3.75	0	0.43	0.24	6.0	0
Patent Wheat White	355	73	13.8	1.5	15	92	0.9	0	0.05	0.03	1.1	0
Enriched Patent Wheat White	355	73	13.3	1.5	100	92	1.3	0	0.35	0.25	1.3	0
Cereals												
Oatmeal	377	64.2	17.5	6.3	60	340	4.0	0	0.8	0.25	1.0	0
Enriched Farina	359	72.8	12.1	1.3	4.8	90	1.3	0	0.345	0.25	1.3	0
Yellow Corn Meal	356	77.7	8.9	1.1	3	140	0.6	500	0.2	0.08	1.0	0
White Corn Meal	359	77	9.3	1.5	4	100	0.7	0	0.14		1.8	0
Rice, Polished	345	79	8.0	0.3	9	96	1.0	0	0.04	0.07	0.9	0
Rice, Unpolished	345	79	8.0	0.3	68	200	2.0	0	0.1	0.08	2.0	0
Wheat Cereal, Whole Grain	350	79	11.4	1.6	30	300	3.7	0	0.6	0.25	2.5	0
Corn Flakes	360	81	8.1	2.0	133	170		0	0.6	0.24		0
Shredded Wheat												0
Fruits												
Apples	64	14.2	0.4	0.5	7	12	0.4	40	Tr	0.02	0.5	10
Apricots	57	13.4	1.1	0	14	25	3.6	2,000	0.03	0.09		Tr
Bananas	99	22.0	1.3	0.6	9	31	1.8	80	0.05	0.03		25
Berries	64	23.8	1.2	0.8	32	30	0.8	300	0.04	0.1		25
Grapefruit	52	12.2	0.6	0.1	21	20	0.3	0	0.07	0.02		40
Melons	40	9.3	0.6	0	17	15	0.5	3,400	0.06	0.07		51
Peaches	41	9.4	0.7	0.1	16	24	0.4	1,000	Tr	0.01		10
Pears	63	14.1	0.6	0.5	15	26	0.5	20	Tr	0.02		5
Pineapples	43	9.7	0.4	0.3	18	28	0.3	200	0.10	0.09		20

Bananas	99	22.0	1.3	0.6	9	32	0.6	210	0.05	0.05		25
Berries	64	23.8	1.2	0.8	32	30	0.8	300	0.04	0.1		25
Grapefruit	52	12.2	0.6	0.1	21	20	0.3	0	0.07	0.02		40
Melons	40	9.3	0.6	0	17	15	0.5	1,400	0.06	0.07		30
Peaches	41	9.4	0.7	0.1	16	24	0.4	1,000	Tr	0.01		10
Pears	63	14.1	0.6	0.5	15	26	0.5	20	Tr	0.02		5
Pineapples	43	9.7	0.4	0.3	18	28	0.3	200	0.10	0.09		20
Plums	85	20.1	1.0	0	20	32	0.8	160	0.50	0.03		5
Prunes (Dried)	300	73.0	2.1	0	54	105	2.9	2,500	0.20	0.06		0
Vegetables												
Asparagus	22	3.3	1.8	0.2	24	35	0.8	900	0.18	1.29	0.5	40
Beans, Green Snap	41	7.4	2.3	0.3	46	52	1.0	700	0.07	0.35	0.6	20
Beans, Lima	120	22.0	7.1	0.7	28	133	11.7	1,000	0.34	0.06		40
Beets, Roots	46	9.7	1.6	0.1	29	39	1.8	200	0.05	0.09	0.3	10
Cabbage	31	5.6	1.6	0.3	45	29	0.3	900	0.08	0.12	0.3	100
Carrots	45	9.3	1.1	0.4	56	46	1.0	7,500	0.07	0.02	0.4	Tr
Cauliflower	30	4.7	1.8	0.5	123	61	1.4	40	0.17	0.02		40
Celery	18	3.3	1.1	0.1	78	37	0.8	50	0.04	0.04		10
Lettuce	19	2.9	1.2	0.3	43	42	1.9	2,500	0.10	0.08	0.5	10
Onions	48	9.9	1.6	0.3	34	45	0.3	6,000	0.03	0.01	0.1	10
Peas, Garden	100	16.9	7.0	0.5	28	127	1.7	1,000	0.4	0.2	1.3	25
Potatoes	85	18.4	2.2	0.1	14	58	0.9	50	0.15	0.10	0.5	25
Spinach	24	3.2	2.1	0.3	67	68	6.6	24,000	0.1	0.08	1.7	50
Sweet Corn	101	19.7	3.1	1.1	6	103	0.5	1,500	0.15	0.17		40
Sweet Potatoes, Yellow	124	27.4	1.8	0.7	19	45	0.9	5,500	0.09	0.13	0.1	30
Tomatoes	23	3.9	0.9	0.4	11	26	0.6	19,000	0.08	0.14	0.4	30
Meats												
Beef, Muscle	205	0	20.6	3.7	12	216	3.0	56	0.39	0.25	7.0	Tr
Fowl, Muscle	220	0	19.3	16.3	16	218	1.4	Tr	0.74	0.14	7.5	Tr
Lamb, Muscle	110	0	19.4	3.7	14	270	4.0		0.38	0.33	8.0	Tr
Pig, Muscle	190	0	19.8	10.6	10	215	2.2		3.95	0.29	7.5	Tr
Sheep, Muscle	190	0	19.8	12.4	15	278	4.0		0.60	0.27	4.1	Tr
Beef, Liver	130	1.7	14.1	4.2	8	373	12.1	24,000	1.20	2.8	25.0	45
Calf, Liver	136	4.0	19.0	4.9	8	373	12.1	12,000	0.52	2.2	25.0	50
Fish and Sea Food												
Lean Fish	50	0	12.0	0.3	12	110	0.5	Tr	0.08	0.18	3.0	Tr
Fatty Fish	130	0	19.0	6.1	21	220	0.8	300	0.09	0.14	5.5	Tr
Lobster	83	0.4	16.4	1.8	75	210	0.4		0.38	0.13	2.7	5
Oyster	50	3.7	6.2	1.2	52	155	3.1	420	0.15	0.46	1.3	Tr

[1] Compiled from various sources [7, 9, 10] and represent average figures for 100 grams uncooked portions, except for meat and fish which represent cooked portions. Cooking losses for fruits and vegetables are considerable for thiamin and ascorbic acid, ranging from 30 to 50 per cent for thiamin and 50 to 100 per cent (usually approaches 100 per cent) for ascorbic acid. Cooking losses in vitamin A, riboflavin, and nicotinic acid are smaller, usually 20 to 30 per cent. In estimating the vitamin content of cooked fruits and vegetables, calculate: thiamin as 50 per cent of fresh value as given in the table; ascorbic acid as nil, except for tomatoes which may be given full value; vitamin A, riboflavin, and nicotinic acid as 75 per cent of fresh value as given in the table.

* Wide variations in values.

Tr.—Trace, calculate as absent, for ascorbic acid values less than 5 mg. per 100.

Grams are recorded as Tr.

Blank spaces—Indicate values not available.

If a higher carbohydrate diet is desired, this can be obtained by increasing the amounts of cereal, bread, or orange juice. This should never be accomplished by increasing the amount of sugar, jam, or jellies. Less fat can be obtained by serving skimmed milk and omitting cream and butter. If less protein is thought advisable, some of the meat, some of the milk, and the whites of eggs may be omitted. It must be remembered that if this is done provision should be made for supplying iron and, if continued for a considerable period of time, calcium. If more protein is desired this can be easily obtained by adding egg whites (each egg white furnished 6 gm.) or lean meat (100 gm. cooked meat furnished 25 gm. protein).

This basic diet should be supplemented at the very beginning, if possible, or later as soon as possible, by increasing the meat ration, of which a good portion should be liver, and by adding a variety of fruits and vegetables, pureed if necessary. Under these conditions, if the caloric requirement is exceeded, modifications in the amounts of bread and cereals can be made. In order to facilitate modification of this diet the essential nutritive composition of the foods most commonly used are given in Table 4.

During any illness lasting more than three days, or from the onset if there is interference with food intake, or if there is increased requirement or excretion, it is necessary to supplement the diet with various nutritional essentials. The amounts recommended here are based on the principle that it is better to err on the side of wasting the vitamins than giving too little. Routinely these patients should be given daily by mouth 25,000 to 50,000 units of vitamin A, and a source of the vitamin B-complex. For the latter such products as 30 gm. of brewer's yeast, 20 gm. of vegex, 45 cc. of aqueous liver extract, or 15 gm. of a yeast concentrate are preferable to synthetic products in capsule form from which some factors as yet impossible to encapsulate are possibly missing. If there is interference with absorption or utilization, or if the requirement is markedly increased, the vitamin A should be increased to 100,000 units or more, and the

B vitamins should be given parenterally. To accomplish this it is advisable to administer daily by intramuscular injection 2 or 3 cc. of crude liver extract (1 cc. equivalent to 1 U.S.P. unit of anti-pernicious anemia factor), to which should be added 10 mg. thiamin, 2 mg. riboflavin, and 100 mg. nicotinic acid amide.

At times the oral administration of bulky sources of vitamin B-complex cannot be tolerated. Under such circumstances crystalline B vitamins should be given twice daily in the following amounts: Thiamin, 2.5 mg.; riboflavin, 3.75 mg.; nicotinic acid amide, 25 mg. If for any reason orange juice cannot be taken, 200 to 300 mg. of ascorbic acid may be given in two doses.

In many patients clinical evidence of nutritional failure is already apparent by the time the patient consults a physician. This should be treated by the same diet, the same supplements by mouth, and the same parenteral injections of crude liver extract. The chief difference in therapy is in giving additionally larger amounts of the vitamin specifically indicated by the signs and symptoms. A guide to the daily dosages of the specific vitamins is given in Table 5. If the patient has liver disease or diarrhea, or is an alcoholic, the larger of the recommended doses should be given. Clinical observation as well as experimental evidence indicate that in these conditions much larger doses of vitamins are required to produce maximal re-

Table 5. Daily dosages of vitamins for specific deficiencies.

Deficiency	Mild	Moderate	Severe
Vitamin A	100,000 U. (m)	200,000 U. (m)	200,000 U. (m)
Vitamin D	2,000 U. (m)	2,000 U. (m)	2,000 U. (m)
Thiamin	10-30 mg. (p)	20-50 mg. (p)	50-300 mg. (p)
Riboflavin	10 mg. (m)	10-20 mg. (m)	20-50 mg. (m)
		10 mg. (p)	10 mg. (p)
Nicotinic Acid	300-500 mg. (m)	500-1,000 mg. (m)	1,000 mg. (m)
or			
Nicotinamide			200 mg. (p)
Ascorbic Acid	500 mg. (m)	500-1,000 mg. (m)	1,000-2,000 mg. (m)
			500 mg. (p)

(m)—by mouth, (p)—parenterally.

sponse than when absorption is unimpaired and the liver normal.

Although the author has never seen hypersensitivity or allergy to vitamins, it is not impossible that such a response will occur, especially on resuming parenteral injections of certain of the vitamins after they have been interrupted (10). Patients giving a history of previous parenteral vitamin medication should always be tested for hypersensitivity prior to resumption of parenteral vitamin therapy. The author routinely tests all patients for the preparation that is to be used.

A Public Health Nutrition Program. The public health program for better nutrition is dependent upon three factors. The first is education, to make people want the proper foods and includes by necessity the teaching of the wise use of income. The second is nutritional improvement of staple foods, in order to surround people with better food. The third is economic, to make more and better food purchasable at every income level.

These three factors are not independent methods of approach but three methods that should be applied simultaneously. For example, the ability to purchase more and better food and the availability of adequate quantities of good food would fail to solve the problem without education to create a desire for these foods. The trilinear approach here outlined requires a coordinated effort of governmental agencies, the medical profession, economists, educators, producers, and consumers.

Education. A clear demonstration of the value of good nutrition should be one of the first methods in the educational program. The demonstration in a community of actual nutritional therapy, such as carried out by Ebbs, Tisdall, and Scott (11) at Toronto on pregnant women not only convinces the physician of its value but by word-of-mouth dissemination from patient-to-neighbor and neighbor-to-neighbor may be of more value than all other educational methods.

The Food and Nutrition Board of the National Research Coun-

cil has already provided the first step in a formal educational program. This was the formulation of daily allowances of the various essential food factors that would constitute a satisfactory daily diet. Although the Technical Commission on Nutrition of the League of Nations adopted nutrition standards in 1935, we have added greatly to our knowledge in this field since then, and the allowances recommended by the Food and Nutrition Board are definitely higher.

Table 6. Recommended daily allowances for specific nutrients.[1] Food and Nutrition Board, National Research Council.

	Calories	Protein	Calcium	Iron	Vitamin A[2]	Thiamin[3]	Ascorbic Acid[3]	Riboflavin	Nicotinic Acid	Vitamin D[4]
		Grams	Grams	mg.	I.U.	mg.	mg.	mg.	mg.	I.U.
Men (70 Kg.)										
Moderately Active	3,000	70	0.8	12	5,000	1.8	75	2.7	18	
Very Active	4,500					2.3		3.3	23	
Sedentary	2,500					1.5		2.2	15	
Woman (50 Kg.)										
Moderately Active	2,500	60	0.8	12	5,000	1.5	70	2.2	15	
Very Active	3,000					1.8		2.7	18	
Sedentary	2,100					1.2		1.8	12	
Pregnancy (latter ½)	2,500	85	1.5	15	6,000	1.8	100	2.5	18	400–800
Lactation	3,000	100	2.0	15	8,000	2.3	150	3.0	23	400–800
Children up to 12 Years										
Under 1 Year[5]	100/Kg.	3-4/Kg.	1.0	6	1,500	0.4	30	0.6	4	400–800
1–3 Years[6]	1,200	40	1.0	7	2,000	0.6	35	0.9	6	
4–6 "	1,600	50	1.0	8	2,500	0.8	50	1.2	8	
7–9 "	2,000	60	1.0	10	3,500	1.0	60	1.5	10	
10–12 "	2,500	70	1.2	12	4,500	1.2	75	1.8	12	
Children Over 12 Years										
Girls 13–15 Years	2,800	80	1.3	15	5,000	1.4	80	2.0	14	
16–20 "	2,400	75	1.0	15	5,000	1.2	80	1.8	12	
Boys 13–15 "	3,200	85	1.4	15	5,000	1.6	90	2.4	16	
16–20 "	3,800	100	1.4	15	6,000	2.0	100	3.0	20	

[1] These are tentative allowances toward which to aim in planning practical dietaries. These allowances can be met by a good diet of natural foods; this will also provide other minerals and vitamins, the requirements for which are less well known.

[2] Requirements may be less than these amounts if provided as vitamin A, greater if chiefly as the provitamin carotene.

[3] One mg. thiamin equals 333 International units; 1 mg. ascorbic acid equals 20 International units (1 International unit equals 1 U.S.P. unit).

[4] Vitamin D is undoubtedly necessary for adults and older children; when not available from sunshine it should be provided probably up to the minimal amounts recommended for infants.

[5] Needs of infants increase from month to month. The amounts given are for approximately 6-18 months. The amounts of protein and calcium needed are less if from breast milk.

[6] Allowances are based on the middle age for each group, and for moderate activity.

The daily allowances of specific nutrients recommended by the Food and Nutrition Board of the National Research Council for various categories are presented in Table 6. Provisions for men are based on various degrees of activity. It should be remembered that this is for an average size man of approximately 70 kg. It is known that larger individuals would require more of these factors. For women, besides the three categories of activity, additional categories of pregnancy and lactation are listed. In these two the allowances are even higher than for a very active man. The allowances for children up to twelve years are listed by various age groups, and above twelve years there are separate categories for girls and boys.

For the education of the public these nutritional allowances, expressed in calories, grams, milligrams, and units, must be converted into practical menus. To meet the dietary allowances set by the Food and Nutrition Board the following regimen is suggested:

> First, eliminate from the diet most vitamin-free or vitamin-poor foods, such as candy, a large part of the sugar (retaining only the amount essential to make the food palatable), jams, jellies, highly-milled grain products that are not "enriched," sweetened carbonated beverages, and alcohol.
>
> Second, include the following in the diet each day:
>
> Milk: For adults, at least 1 pint; for children, 1 quart; a quart a day for all would be better.
>
> One egg.
>
> Meat: 1 serving at least 100 gm. for an adult.
>
> Vegetables: 2 servings, 1 of which should be green or yellow.
>
> Fruit: 2 servings; 1 of these should be a citrus fruit, and the other tomato, apple, or prunes.
>
> Potatoes: 1 or more servings.
>
> Butter, or fortified oleomargarine: 1 to 4 oz.
>
> Whole grain cereal: 1 serving.
>
> Whole grain or enriched bread: 2 to 10 oz.

The balance of the calories, sufficient to furnish the energy requirement, should then be made up from natural or "enriched" food products.

It is impossible within the scope of this chapter to present a detailed dietary list for each of the categories. However, if the caloric requirement of adults and children over twelve years of age is met according to this recommended scheme there is little or no danger that the allowances recommended by the Food and Nutrition Board will not be met. This does not apply to women during the latter half of pregnancy and during lactation. In these two categories, in order to supply the recommended amounts of calcium and iron, ascorbic acid, thiamin, riboflavin, and nicotinic acid it is essential that this basic diet should include at least a quart of vitamin D milk; that the meat ration be not below 200 gm. a day; and that an additional 4 ounces of citrus fruit be provided.

It is recognized that these recommended daily allowances must be translated into many diets. These diets must take into consideration the availability of foods in different sections of our country and at different seasons, and the racial and religious dietary customs of many groups. Above all, each one of these diets must represent to the group which it is to serve a pleasurable experience.

For the successful promotion of this educational program it will be necessary to utilize all the agencies and methods that we now have available. These include our teachers, extension workers in the department of agriculture, home economists, the public health services, the women's clubs, labor, and industry. The methods to be utilized include: first, by example, through serving nutritionally satisfactory noonday meals to children in schools and to workers in industry; second, the teaching of proper nutrition in the schools, from kindergarten to college; and third, education of adults, this to include fathers as well as mothers. All methods of education should be used for all of the groups; that is, assigned lessons and reading, lectures, posters, press, radio, and motion pictures.

Of no less importance is education in the recognition of the early symptoms of nutritional depressions. Laymen should be taught that in infants and children these early symptoms include lack of appetite; failure to eat an adequate breakfast; failure to gain weight steadily; delay in learning to sit, stand, or walk; inability to sit or pain on sitting and standing; aversion to normal play; chronic diarrhea; poor sleeping habits; backwardness in school, and frequent colds. The following physical signs may also be emphasized to lay groups as suggestive of early nutritional deficiency states in children: Lack of subskin fat, paleness, hemorrhage of the newborn, and sores in the angles of the mouth. Public health nurses should be instructed, in addition, to observe children and infants for wrinkling of the skin on light stroking, poor muscle tone, rough skin (toadskin), bad posture, nasal blackheads and whiteheads, rapid heart, red tongue, square head, enlarged wrists and beading of ribs, Vincent's angina and thrush.

The symptoms of nutritional depressions in adolescents and adults that may safely be emphasized to lay groups include lack of appetite, lassitude and chronic fatigue, loss of weight, lack of mental application, loss of strength, sore mouth or sore tongue, chronic diarrhea, nervousness and irritability, paraesthesias, night-blindness, photophobia, burning or itching of the eyes, lacrimation, muscle and joint pains, sore bleeding gums, and tendency to bleed. The signs of which lay groups should be made aware are sores at the angles of the mouth, spongy bleeding gums, and spontaneous hemorrhages into the skin. Public health nurses should be taught to observe also for the following signs in adults and adolescents: Nasolabial sebaceous plugs, cheilosis, glossitis and stomatitis, and facial butterfly, Casal necklace and perineal, scrotal, and vulval dermatitis.

Physicians should be made increasingly aware of the importance of the signs and symptoms enumerated as possibly indicative of nutritional depressions, and in addition should look for vascularize-

tion of the cornea; xerosis conjunctivae; Vincent's stomatitis; minimal changes in color or texture of the tongue such as red, swollen lingual papillae and papillar atrophy; muscle tenderness in the extremities; poor muscle tone; loss or impairment of vibratory sensation in the toes; plantar dyethesia advancing to a sock distribution; changes in the tendon reflexes with particular emphasis on loss of the ankle jerks; skin lesions of pellagra; nonspecific vaginitis; follicular hyperkeratosis of extensor surface of the extremities, and rachitic deformities.

Enrichment of Staple Foods. The Council on Foods and Nutrition of the American Medical Association (12) has established two principles for the improvement of staple foods. 1. In the processing of foods every effort should be directed to retaining in the product the food values of the natural foods from which they are made. 2. For those processed foods which are not nutritionally equivalent to the original food from which they have been made it is in the interest of the public to restore certain dietary essentials so that these processed foods have the full nutritive value of the natural foods with respect to the substances added.

Since wheat and sugar form approximately 55 per cent of the average caloric intake of the American public, it is natural that these two foods should receive first attention. The Food and Nutrition Board of the National Research Council has already recommended the enrichment of flour and bread, and the United States Food and Drug Administration has published a proposed definition and standards for "enriched" flour (8). These standards are presented in Table 1, which also shows the composition of whole wheat and of white flour made from that wheat.

The essential ingredients of an "enriched" flour are thiamin, riboflavin,[8] nicotinic acid, and iron. The optional ingredients for enriched flour include calcium and vitamin D. Although wheat is not a good source of calcium, containing only 240 mg. per pound,

[8] Riboflavin may be made an optional ingredient because of procurement difficulties.

if claims for enrichment with calcium are to be made, flour must contain at least 500 mg. and a maximum of 2,000 mg. per pound. This high figure for enriched flour was adopted because large amounts of calcium are now often added to flour for the quick-rising breads. The whole wheat contains no vitamin D; but since it is a satisfactory carrier, the Food and Drug Administration has permitted an optional enrichment with 250 U.S.P. units of vitamin D and a maximum of 1,000 units. A nutritionally satisfactory flour, meeting the standards for an "enriched" flour, may be obtained in either of two ways: 1, by milling so as to retain most of the vitamin and mineral content of the whole grain; and 2, by adding vitamins and minerals to highly-milled flour.

Various methods can be used in making an enriched bread: 1, by the use of whole grain or enriched flour; 2, by adding the vitamins and minerals to the dough; and 3, by the use of high vitamin and mineral yeast. It is very important that enrichment should not be limited to commercial baked goods but that all flour for home consumption should be enriched, since about 50 per cent of the flour manufactured in our country is converted into foods in the home.

Pertinent criticism is that flour and bread, if enriched by the synthetic substances, are not enriched by other fractions of the vitamin B-complex which are also in many instances known to be removed in the processing. Those who would not recommend the use of enriched bread unless the whole vitamin B-complex can be added must, however, assume the position that good and better are the enemies of the best. All the authorities who recommend the enrichment program recognize that whole-grain flour, bread, and cereals are preferable. But they are not willing to forego providing a better product than is now in use simply because it is not the best possible. Practicability must always be one of the first considerations in any program towards improvement of the public health. In addition, it is believed by many that "enrichment" of white flour and bread will lead to an increased consumption of whole-grain

flour and bread since the necessity for the "enrichment" points out the value of the whole grain products.

The importance of the enrichment program is evident if one studies the plan developed by Carpenter and Stiebeling (13) for adequate diets at four different levels of cost. The weekly allowances of flour and cereals for the moderately active man of average weight are 5 lb. 4 oz. in a restricted diet for emergency use; 4 lb. 8 oz. in a good diet at minimum cost; 4 lb. in an adequate diet at moderate cost; and 2 lb. 4 oz. in a liberal diet. These amounts are those customarily used by many families reported in the various Bureau of Home Economics surveys. The use of "enriched" in place of ordinary refined bread, flour, and cereals would result in significant increases in iron, thiamin, riboflavin, and nicotinic acid intake in these diets. For example, in the emergency diet the amount of thiamin would be increased by 456 I.U.; in the good diet at minimum cost by 390 I.U.; and in the liberal diet by 194 I.U. per person daily. It should be emphasized, however, that those who eat only from one to three slices of bread daily would not receive significant additional amounts of vitamins and minerals.

The Price-Rationing Factor. It is not the function of the physician to point out to the political, agricultural, and industrial economist the means by which more and better food, sufficient to provide a nutritionally satisfactory diet at all living levels, may be made available. It is his function only to point out the existence of this factor as an integral part of the whole problem.

Conclusion

The nutritional status of large groups of the American people represents a challenge not only to the medical profession and public health workers but to the entire nation. For millions of people to be subsisting on diets providing less than the satisfactory levels necessary for good nutrition, is a national disgrace in peace time and a national danger in an emergency.

Our newer knowledge of nutrition, accumulated in the last thirty years, enables us not only to understand the nature of the problem but also to devise effective measures towards solving it. Fortunately, both governmental agencies and scientific organizations have already launched a well-conceived program which gives promise of improved nutrition, better health, and more meaningful living to the American people now and in the future. This is written without undue optimism, and with the understanding that the most far-reaching effects of this program may only make themselves felt in generations rather than years.

It remains for all who are concerned with the public welfare, the leaders in the various fields of culture and endeavor, to give this program their material support in order to win the cooperation of all the people towards the accomplishment of this high purpose.

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THE RELATION OF EMPLOYMENT LEVELS TO BIRTHS IN GERMANY[1]

DUDLEY KIRK

SINCE 1933 Nazi Germany has made an organized effort to raise the German birth rate, and the success of this positive population policy is evidenced by a notable rise in births prior to the present war. The birth rate, which had declined to 14.7 per 1,000 in 1933, had risen to 20.3 in 1939. The latter is not a particularly high birth rate and represents a fertility only slightly above that required for permanent replacement of the population. But because fertility was so low before, the rise is an important one. In absolute terms there were 436,000 more births in the Old Reich in 1939 than in 1933. In the German-speaking areas of the Greater Reich the increase was about 500,000. The comparative magnitude of this increase is suggested by the fact that there were only 612,000 births in France in 1938, 67,000 less than in 1933.

A number of students of population, including Whelpton, Hankins, Glass, and the Taeubers, have appraised the German population effort with some care.[2] All of these writers have pointed to the difficulties of sorting out the effects of specific policies, such as marriage loans, grants to large families, family allowances, suppression of abortions, etc., as over against other factors influencing fertility. It is the purpose of this article to evaluate one of the most important of the "other factors," namely, employment conditions. Also, it is hoped that certain of the conclusions may have more general ap-

[1] From the Office of Population Research, Princeton University, in cooperation with the Milbank Memorial Fund.

[2] Whelpton, P. K.: Why the Large Rise in the German Birth Rate? *American Journal of Sociology*, November, 1935, xli, No. 3, pp. 299-313; Hankins, Frank H.: German Policies for Increasing Births. *American Journal of Sociology*, March, 1937, xlii, No. 5, pp. 630-652; Glass, David V.: POPULATION POLICIES AND MOVEMENTS IN EUROPE. Oxford, 1940, pp. 269-313; Taeuber, Conrad and Irene B.: German Fertility Trends, 1933-39. *American Journal of Sociology*, September, 1940, xlvi, No. 2, pp. 150-164.

plicability regarding the relations between economic conditions and births.

Employment indices, as opposed to other economic data, were selected for a number of reasons. Among the most important is that the condition of employment or unemployment is a fact of immediate and decisive importance in people's lives. As a motivating factor, it is probably far more important than diffuse concern about rising costs of living, annoyance over increased taxes, or even the fear and the fact of wage reductions. These things pale by comparison with the personal and familial calamity of losing one's job without much hope of getting another one.

A conclusive reason for using this index for the purpose at hand is the fact that in Nazi Germany other economic indices of significance to population trends, such as wages, cost of living, etc., remained practically constant, while births rose. On the face of things, these other economic variables could not have been important direct influences on fertility. They would be such only if constancy itself tended to increase the number of births through the economic security that predictability suggests. Under a revolutionary regime that stressed turmoil and change, it would be surprising if this were the case.

In contrast with wage and price changes, changes in employment were enormous. When Hitler became Chancellor in 1933 there were fewer than 12,000,000 employed persons and about 6,000,000 unemployed. At the outbreak of war in 1939 there were about 21,500,000 employed and about 40,000 unemployed in the same territory; that is, practically all the unemployed were reabsorbed into the economy and about 4,000,000 more were drawn from other sources, including immigration from abroad.

It should be noted in passing that these figures do not relate to the total labor force, but to persons covered by the sickness insurance system, which includes practically all wage employees. This system does not include peasants, many artisans and professional people,

entrepreneurs, and persons in the higher income brackets. It does include persons in labor camps, but not members of the armed forces. That is, the increase in the size of the Germany army was over and above the increase in the number employed. For "Folk" Germans employment opportunities in Hitler's Germany were much superior to what they had been in the latter years of the Republic. Also, they were better than in neighboring countries, judging by the fact that there was a net immigration of 500,000 persons between 1933 and 1939. This was in excess of the much more publicized out-migration of refugees. It does not include organized migration of the Baltic and other Eastern Germans to the Reich.[8]

The impact of this reemployment on the economic outlook of the German people must have been very important. A comparable achievement in the United States would have involved the reemployment of perhaps 20,000,000 persons between 1933 and 1939. It is unbelievable that such an increase in employment would have had no effect on fertility.

In order to carry out an organized attack on the problem of the influence of employment on births, a set of *a priori* hypotheses was formulated to be tested by whatever statistical evidence was available. The hypotheses were:

(a) In the short run, changes in the birth rate are closely associated with employment levels. Employment conditions are not an independent cause of long range secular trends in birth rates, but they may be decisive in determining short range fluctuations.

(b) The relationship should have increased as fertility declined in time.

(c) The relationship should be greater in urban and industrial regions and countries than in rural regions and countries.

Considering the first of these, it seems rather evident that the secular trend of the birth rate could not be associated positively with

[8] On the basis of census data for Austria and the Sudetenland, it seems likely that the bulk of the immigrants into Germany between 1933 and 1939 were German-speaking persons from these areas.

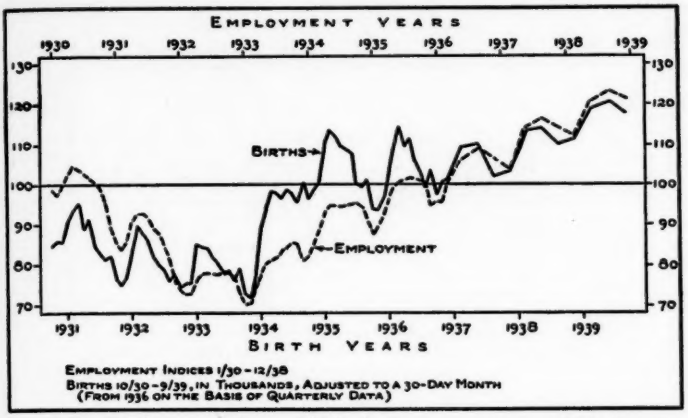

Fig. 1. Employment indices and births in Germany, 1930-1939, births lagged nine months.

employment conditions, because over any considerable time period, birth rates have declined and employment has not. To check the influence of employment over shorter periods, a series of correlations of employment indices and births by months and by quarters was made, using data from various countries and for various time periods. Experimentation revealed that the highest correlations were achieved where births lagged nine months, or three-quarters, behind employment, depending on the nature of the data. The correlations in Germany were generally high.

Figure 1 presents the monthly fluctuation of employment indices and births from 1930-1939, with births lagged nine months. The correspondence of the two is clear throughout, though the rise of births much outdistanced the rise in employment in the first years of Nazi control. Over the whole period the correlation was +.79 for quarterly data.

A first question obviously arises as to whether the apparent high correlation was merely the result of the chance concatenation of basic trends. The association of employment and births in the early 'thirties might well be a fortuitous relationship arising from the

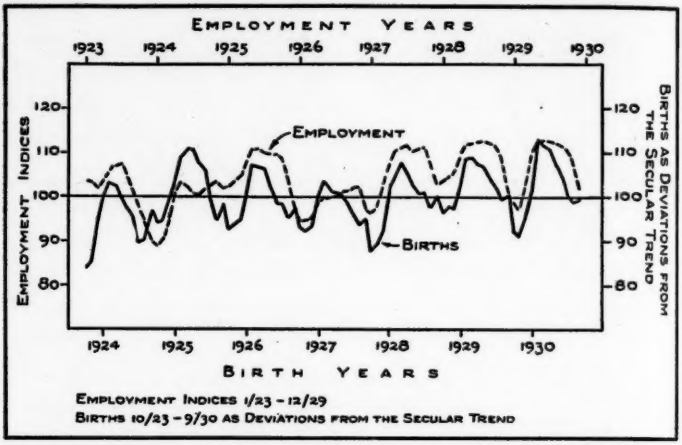

Fig. 2. Employment indices and births in Germany, 1923-1930, births lagged nine months with secular trend removed.

effects of depression conditions on employment and the quite unrelated continuation of the downward secular trend of births. In the later period the rise of employment and of births might well be the result of completely independent aspects of Nazi policies. As is well known the degree of correlation in time series may be greatly magnified by such coincidence in trends.

To test this possibility, reference was made to the relationship between employment and births in the period from 1923 to 1930. The correlations in the earlier period were not of a very high order, but they were statistically significant. However, when the downward secular trend of births was removed, as in Figure 2, the coefficients were found to be quite high. This was especially true of the period after 1926. The association was weak in the earlier years, perhaps in part because of the vagaries of inflation and its aftermath, which so disturbed the German economy through 1923 and 1924. The coefficient of correlation in the period 1923-1926 was only $+.37$, as compared with $+.88$ in the period 1926-1929, and $+.62$ over the entire series.

Fig. 3. Employment indices and births in Germany, 1923-1930, adjusted for seasonal variation, births lagged nine months with secular trend removed.

This chart also affords something of a test of the second hypothesis, namely, that the relationship between employment and births should have increased as fertility declined in time. The relationship in the early part of the period was somewhat tenuous, later increasing and rising to a rather impressive association in the latter part. However, as has been indicated, the early period was much disturbed by inflation and this test is not completely adequate, though what little evidence there is accords with the hypothesis. The testing of this hypothesis in other countries was generally hampered by the difficulties of getting coherent employment series for periods before 1930.

It is apparent in the entire series from 1923-1940 that seasonal variation of employment and births (lagged nine months) coincide and that this fact might possibly explain much of the correlation noted. When seasonal variation in addition to secular trend is removed, as in Figure 3, there is clearly no significant correlation in the earlier 'twenties. Births reacted at once to the stabilization of the Reichsmark in November 1923, whereas employment rose

EMPLOYMENT YEARS
1930 1931 1932 1933 1934 1935 1936 1937 1938
EMPLOYMENT INDICES AND BIRTHS
130 120 110 100 90 80 70
MARRIAGES
65 60 55 50 45 40
MARRIAGES
BIRTHS
EMPLOYMENT
1931 1932 1933 1934 1935 1936 1937 1938 1939
BIRTH YEARS
EMPLOYMENT INDICES 1930-1938
MARRIAGES 1930-1938, MONTHLY AVERAGES IN THOUSANDS
BIRTHS, LAST QUARTER 1930 - THIRD QUARTER 1939, MONTHLY AVERAGES IN THOUSANDS

Fig. 4. Employment indices, marriages, and births in Germany, by quarters, 1930-1939, adjusted for seasonal variation, births lagged nine months.

more slowly. When employment did rise, births failed to respond. Between 1926 and 1930 there was a close correspondence in the movements of the two variables despite adjustment for seasonal variation, and the correlation was high, namely +.77. The correlation in this period indicates that the relationship is not a transitory one that might have arisen in the 'thirties owing to a chance association of trends.

The coefficient of correlation in the period from 1930-1939 was somewhat higher for the adjusted data (+.83) than for the uncorrected data presented in Figure 1, indicating that the relationship of the variables was independent of seasonal fluctuation. Another factor, however, clearly disturbs the relationship in this period, namely marriages. The correlation coefficient for marriages and births was +.72, indicating a degree of interdependence almost as great as between employment and births. Examination of Figure 4 will reveal that fluctuations of births often appear to be a compromise between changes in employment and in marriages. The great increase in births in 1934 in particular seems to have been as much influenced by marriages as by employment, no doubt as a

result of the official policy of encouraging marriages through marriage loans without interest. In the later period the pull of reemployment seems to have been greater. The logic of this explanation is upheld by the fact that the initial rise of births in Germany was mostly a function of first births, whereas in the later period rises in fertility were brought about by increases in the higher orders of parity, especially in second and third births. When marriages were held constant the correlation coefficient between employment and births rose to +.90 for the period 1930-1939.

Surprisingly enough, the number of marriages does not appear to be very closely linked to employment conditions. The coefficient of correlation for the period 1930-1939 was only +.33. However, the decline in marriages contrary to employment trends in 1935 may be explained by the fact that the high marriage rates of late 1933 and 1934 could not be indefinitely maintained, owing to the limited number of marriageable persons available once the marriages postponed from the depression years were consummated.

According to the last of the three hypotheses, the relationship between employment and births should be greater in urban and industrial regions and countries than in rural regions and countries. On *a priori* grounds this should be true (1) because the impact of employment conditions in rural areas is cushioned by the high proportion of peasants and small town merchants and craftsmen, (2) because more rational control of fertility is to be expected in the city than in the country.

Within Germany births in large cities appear to be more responsive to changing employment conditions than in the country as a whole, particularly in periods of declining employment. However, large cities seem to have been more affected by the rapid increase of the marriage rate than the rural areas, and births rose more rapidly in the cities than in the remainder of the country in the first years of the Nazi regime. Employment also rose more rapidly in these areas.

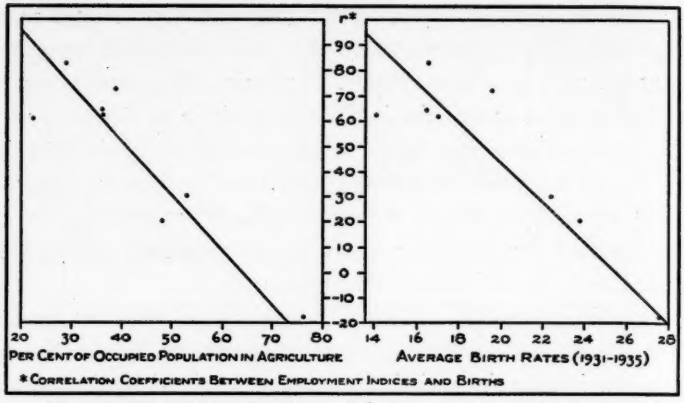

Fig. 5. Relation of r in selected countries[1] to per cent of occupied population in agriculture and to birth rates.

[1] The coefficients of correlation and time periods to which they apply were: Germany +.83 (1930-1939); Czechoslovakia +.72 (1931-1937); France +.64 (1930-1935); Sweden +.62 (1933-1938); United States—A. F. of L. data— +.61 (1935-1939); Hungary +.30 (1933-1940); Italy +.20 (1929-1936); and Poland —.18 (1931-1938). Except for the United States the proportions of the occupied population in agriculture were obtained from the League of Nations. EUROPEAN CONFERENCE ON RURAL LIFE 1939. POPULATION AND AGRICULTURE WITH SPECIAL REFERENCE TO AGRICULTURAL OVER-POPULATION, p. 8. Birth rates were taken from *Population Index*, July, 1941, vii, No. 3, pp. 244-245.

Comparisons between countries reveal that there is a negative relation between the proportion of the occupied population in agriculture and the association between births and employment. In Figure 5, the correlation coefficients between employment and births are plotted against percentage of the working force in agriculture. The countries and time periods were selected on the basis of the availability of the data.[4] Because of the character of the data and the varying time periods used the precise relationships between

[4] The coefficients were computed, as for Germany, with births lagged nine months. Employment indices were taken from various issues of the *International Labour Review* and birth data from official sources of the countries concerned. Great Britain was omitted because its employment series in certain respects is noncomparable with those used. On the basis of the British employment figures as given and without adjustment for secular trend of births, there is a negligible correlation between employment and births. When the secular trend and seasonal variation of births are removed (assuming the years 1924-1931 to represent the true secular trend) the correlation between employment and births from 1924-1938 was +.86.

countries are of little significance. However, the general pattern is clear. Poland, Italy, and Hungary, with relatively high proportions of their populations in agriculture, had low or even negative associations between employment and births. In contrast, the more industrial nations of Western Europe generally had significant correlations between the two variables.

The pattern is equally distinct as regards the level of birth rates, also presented in Figure 5. Countries of high birth rates, notably Poland and Italy, had no significant relation between employment and births. Countries of low birth rates, such as Sweden, France, Germany, and the United States, had a rather close association.

In general the *a priori* hypotheses on the relationship of employment and births were supported by the data. The high degree of covariation in Germany therefore fits into a logical scheme that greatly strengthens the purely statistical evidence. Both logic and statistical evidence bear out the belief that employment must have been an important element in the rise of the German birth rate.

A final series of evidence may serve to strengthen the argument and give some quantitative estimate of the importance of employment conditions. Examination of changes in employment levels and birth rates between 1932-1933 and 1938-1939 suggests that a large increase in births in Germany might well have been anticipated on the basis of reemployment alone. In Figure 6 these changes are presented for those countries demographically comparable[5] to Germany for which data on general employment levels are available. The countries are arranged from left to right in order of the ratio of employment in 1938 to employment in 1932. These columns are paired with similar ratios for birth rates in 1939 as compared with 1933.

Germany experienced the greatest increase in employment and at the same time much the greatest rise in the birth rate. Sweden,

[5] Though employment series were available for certain Eastern European countries, they were not included. In these predominantly agricultural countries of high fertility, birth rates continued to decline regardless of changes in employment conditions.

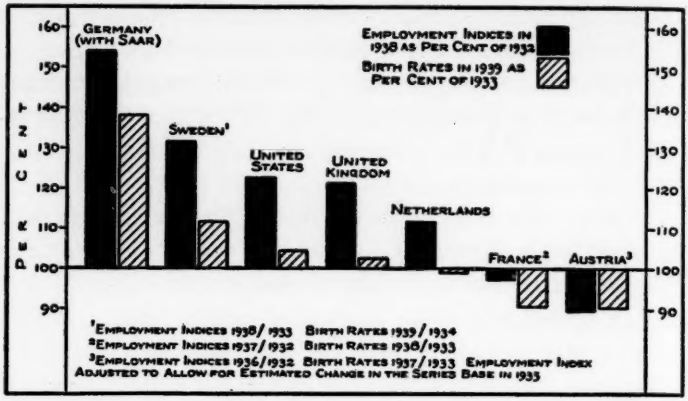

Fig. 6. Changes in employment indices and birth rates.

and to a lesser extent the United States and Great Britain, also achieved considerable reemployment with some recovery in births. In The Netherlands, where reemployment amounted to only 12 per cent, the birth rate remained about constant, and in France and Austria employment and births both declined in the period.

In every country except Austria the position of employment in the later year relative to the earlier was better than that of births. This fact tends to confirm the frequently expressed opinion that the checking and even reversal of birth rate declines in the late 'thirties does not represent a true deviation from the downward secular trend but a temporary reaction to improved economic conditions. Among the countries considered a certain increase of employment was necessary to hold the birth rate constant, and barring such increase, birth rates tended to pursue their downward course. In Sweden, for instance, a 32 per cent increase in employment produced a rise of only 12 per cent in the birth rate; in the United States a 23 per cent employment rise brought a birth rate increase of only 4 per cent; and in The Netherlands a 12 per cent rise in employment was not quite sufficient to hold the birth rate at its former

level. In France and Austria, where employment conditions worsened, the downward trend of births continued.

In so far as Germany is concerned, it seems certain that on the basis of comparative experience an important rise in the birth rate could have been predicted from reemployment alone. At least a third of the birth increase may be assumed to be a function of reemployment, since in Sweden, with far less increase in employment, the birth rate rose almost one-third as much as it did in Germany. On the other hand, if the rise in births followed the rise in employment at the same distance as it did in Sweden (*i.e.* 20 per cent), almost all of the rise in German births would have to be assigned to the improved employment situation.

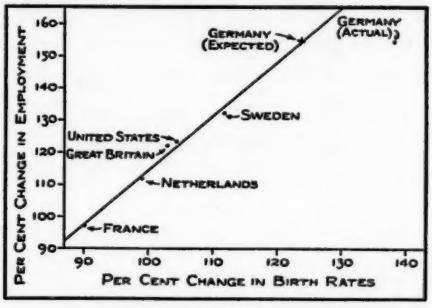

Fig. 7. Changes in birth rates relative to changes in employment indices.

The experience of other countries demonstrates that the latter interpretation is unlikely. There is clear evidence that, at least among the countries considered, successive improvements in employment conditions did not bring about a proportionate rise in births. The spread between changes in employment and in birth rates broadened as the employment ratio rose. Thus the spread was only 7 per cent in France, 13 per cent in The Netherlands, 19 per cent in Britain, 18.5 per cent in the United States, and 20 per cent in Sweden.

The graphic presentation of the relationship between changes in employment and birth rates in these five countries (Figure 7) reveals a striking regularity in the diminishing influence of employment on births as the employment ratio goes up. This is indicated by the relatively small deviations of the plotted points from the

computed line of regression. With employment changes known, the change in the birth rate in any one of these countries could have been estimated with reasonable accuracy from the experience of the other four. The experience of these typical countries suggests a high degree of predictability in the association between employment and birth rates in Western industrial nations generally.

Assuming that Germany reacted to changing employment conditions as did these other Western countries, a 23 per cent rise in the birth rate could have been expected from reemployment alone.[6] The actual increase was 38 per cent. On this basis almost three-fifths of the actual increase may be explained by reemployment, leaving only two-fifths to be allocated to other changed conditions in the Reich, including all direct population measures.

Most will agree that, behind the Nazi façade of collective expressions that are so objectionable and so dangerous, the Germans are human beings with hopes and fears and with reactions to stimuli not unlike our own. If this is true, a major part of the Nazi successes on the demographic front must have been the result of reemployment, as opposed to the more spectacular appeals of race and "folk" on the one hand, and specific inducements to childbearing on the other.

[6] This percentage may be obtained by reading the expected increase in the birth rate at the actual employment ratio from the extrapolated line of regression in Figure 7.

MEDICAL EVALUATION OF NUTRITIONAL STATUS[1]

IX. THE RELIABILITY OF VISUAL THRESHOLD DURING DARK ADAPTATION AS A MEASURE OF VITAMIN A DEFICIENCY IN A POPULATION GROUP OF LOW INCOME

ELEANOR P. HUNT, M.D. AND KENNETH M. HAYDEN

SERIAL dark adaptation measurements of adults of low income are described in the present report. The measurements represented one aspect of a larger inquiry into the utility of visual threshold measurements in the appraisal of subclinical vitamin A deficiency. The dark adaptation studies referred to were part of the Cooperative Nutrition Study, the purpose and scope of which have been discussed elsewhere (23). The measurements now reported were made to ascertain: 1, The effect upon visual threshold of vitamin A therapy administered to approximately one-half of the subjects; and 2, the extent of variations in threshold at specified dark times[2] which were not due to therapy or to instrumental factors. The threshold variations to be described are errors of measurement at different levels of experience with the adaptation test and temporal variations of the individual's nearly dark adapted threshold during a six-week period. Quantitative information dealing with such variations is scant. With reference to the adaptometer (14), the instrument used, errors of observation and day to day variation have been commented upon in three reports (15, 27, 22). Errors of single measurements of inexperienced subjects were described in an earlier report from the Cooperative Nutrition Study (17).

[1] This paper is the ninth of a series from a cooperative investigation by the Cornell University Medical College, Department of Public Health and Preventive Medicine and Department of Pediatrics; Milbank Memorial Fund; the New York City Department of Health; and the United States Public Health Service, Division of Public Health Methods, National Institute of Health.

The cooperating agencies have been assisted in carrying out this investigation by the Work Projects Administration for the City of New York, Official Project No. 65-1-97-21 W.P. 24, "Medical Evaluation of Nutritional Status."

[2] The time (minutes) elapsed in the dark following light adaptation.

Material and Methods

The Sample. Sixty men and twenty-three women were the subjects selected for study. The age range of the group was 20-65 years. The only criteria of selection were willingness to participate in the study and employment as a project worker certified to receive public assistance. The group represented a low-income level. Expenditures for food were necessarily small and the daily provision of vitamin A in the diet was accordingly believed to be below recommended allowances for adults (31). Subsequent gross and biomicroscopic examination of the eye revealed that signs of vitamin A deficiency, either in the form of thickening and opacity or marked translucency of the conjunctiva were present in 72 per cent of the subjects who participated throughout this study. A report of the biomicroscopic examinations has appeared elsewhere (24).

Apparatus. Three adaptometers were used. The operating current of each instrument, controlled by a rheostat and voltage regulator assembly, was adjusted to provide a preadaptation brightness of 1,004 millilamberts. Calibrations of the preadapting lights were made with the Macbeth illuminometer immediately before the initial tests. The National Bureau of Standards checked the manufacturer's calibrations of wedges, "neutral" filters, and chromatic filters for two instruments. The corresponding parts of the third instrument were calibrated by the manufacturer only. Results with this instrument were comparable to those of the other instruments when the same subjects were examined.

The adaptometers were operated in a suitably constructed dark room. The preadapting light and the test flash were viewed 7 degrees nasally, using the right eye. The area of the retinal field during preadaptation for 3 minutes was approximately 35 degrees. During dark adaptation the test field occupied a retinal area 3 degrees in diameter. The duration of the test flash was .2 second. The violet filter (Corning 511) was used during threshold determinations to transmit wave lengths below 460 millimicrons. Brightness of threshold was expressed in the logarithm of micromicrolamberts (log $\mu\mu l$).

Procedure. The tests were made by three technicians who were uniformly and thoroughly trained by one senior field technician of the

United States Public Health Service. Each of the technicians had tested approximately 300 subjects in the Cooperative Nutrition Study before the present series of tests was made. All tests of one subject were made by the same technician with one adaptometer.

Threshold was defined as the least brightness perceptible to the subject. The subject's oral response to this critical brightness was "yes" (seen). To slightly less brightness than this critical value the oral response was "no" (not seen). The critical brightness was found by means of a sequence of test flashes in which brightness was increased following the "no" response, and decreased following the "yes" response until alternating responses were obtained at relatively narrow limits of brightness.

Measurements of threshold were made within each of twelve approximately 1-minute intervals equally spaced during dark adaptation. These observation intervals were centered at the following dark times: 0.5, 3.5, 6.5, 9.5, 12.5, 15.5, 18.5, 21.5, 24.5, 27.5, 30.5, and 33.5 minutes. To obtain the threshold within a given observation interval, the technician began the flash sequence about 30 seconds or more before the earlier limit of the interval so as to bring the subject near the stage of alternating "no" and "yes" responses to small changes in brightness before the interval was reached. The threshold was then finally determined at or near the midpoint of the interval and the actual observation-time was recorded to the nearest 5 seconds. A multiple correlation analysis of threshold, dark time, and number of flashes in a large number of flash sequences showed no relation between the threshold obtained and the number of flashes employed.

Duplicate dark adaptation tests were made in the following manner: the first test was completed in about 40 minutes (3 minutes light adaptation and 33-34 minutes dark adaptation) and the subject rested for 20 minutes in office illumination. The subject was then light adapted again and a second dark adaptation test was made by the same technician. In the second test, threshold in each of the twelve observation intervals was measured exactly as in the first test. The record of the first test was collected from the dark room by a clerk before the second test was begun. In all observation intervals the actual observation-time at which the threshold was determined was very nearly the same in the first and second tests of any duplicate pair. A study of the differences between actual observation-times in the first and second tests respectively, at

each interval, showed that 1, the mean difference in time was zero, 2, the standard deviation of the difference was less than ±.25 minute. Because of the close correspondence in actual observation-time, thresholds in the first and second tests of a duplicate pair, within any observation interval, were regarded as directly comparable.

Schedule of Examination and Therapy. During the study period of six weeks duplicate tests of all subjects were scheduled at each of four examination periods. Preliminary to the arrangement of the examination schedule the subjects were classified according to age (less than 40 years of age, 40 years and older) and sex. The schedule was then set up so that the several technicians tested approximately equal numbers of subjects drawn at random from the subjects in each age-sex group.

Ten work days, or two calendar weeks, were required by each technician to complete duplicate tests of the subjects assigned. These two weeks constituted an examination period. The order of testing the subjects in the first period was maintained with minor exceptions at each of the three later periods. Thus the interval between repeated tests of the same subject was regularly two weeks and at all examination periods a given subject was tested at the same time of day.

The entire group of eighty-three subjects was tested in duplicate at the first examination period. For a number of reasons all of these subjects could not be retested at each of the three examination periods which followed. For example, certain subjects were transferred to another work-relief project. Others discontinued tests because of lack of interest or inability to be released from office work at the necessary hours. Fifty subjects were examined in duplicate at each of four examination periods and thirty completed duplicate tests at less than four periods.

Subjects who were to receive vitamin A therapy were designated when the examination schedule was arranged by selecting by a random procedure approximately one-half of the cases from each of the subgroups resulting when the subjects were classified according to age, sex, and technician. Of the fifty subjects who were examined at four periods, thirty received vitamin A therapy and twenty served as controls. The case record of treatment for the thirty subjects in the therapy group showed that the supplement was taken 90 per cent or more of the days during the study by twenty-one of these subjects. Seven others in this group of thirty had therapy 80-89 per cent of the study period and the

remaining two subjects had therapy 76 and 78 per cent respectively of the total days under observation.

Therapy was begun immediately after the initial duplicate tests in examination period I. Each subject in the therapy group was given daily one Afaxin capsule containing 10,000 International units of vitamin A. The supplements of vitamin A had the effect of increasing the dietary supply of this vitamin to a level of intake which was well above the recommended daily allowance for adults. On week days the capsules were dispensed by a clerk in the laboratory and swallowed in his presence. Capsules to be taken at home on Saturdays and Sundays when the laboratory was closed were delivered to the subjects on Friday afternoons. A report on the use of the week-end supply was required Monday mornings.

Results

Individual Trends of Threshold in the Therapy and in the Control Groups. Measurements at 33.5 minutes in the dark were selected for a comparison of change in threshold in the therapy and control groups respectively, during the six weeks. The selection of a threshold late in adaptation was made because: 1, immediate errors of measurement, which will be discussed in a later section, were smaller than at earlier dark times; 2, the nearly dark adapted threshold approximates a cumulative measure of the secondary phase of adaptation (10); and 3, such thresholds have been found to be more responsive than earlier ones to changes in the dietary supply of vitamin A (3, 26, 44, 15).

The trend of threshold at 33.5 minutes was measured for each of the fifty subjects, who were tested in duplicate at each of four examination periods. An individual linear regression of the threshold at this dark time on days elapsed during the study was used for this purpose. The individual regression coefficient, or the slope of threshold, was taken as a measure of the consistent day to day change in threshold (log $\mu\mu l$ per day). Each coefficient was derived on the eight observations of the subject obtained in duplicate tests at four examination periods.

If supplements of vitamin A had the effect during the study period of lowering threshold at 33.5 minutes, one would expect negative individual slopes to occur more frequently in the therapy group than in the control group. The summary of individual slopes in Table 1 provides no evidence of such a difference in the present study. Negative slopes were found with about equal frequency in both treatment groups. For example, for the nine subjects in the

Table 1. Individual slope of threshold at 33.5 minutes (dark time) during six weeks for subjects in the therapy and in the control groups who had significant trends, and the number of cases in each group for whom the individual slope did not differ significantly from zero.

Item	Individual Slope (b)[1]													
	Therapy Group							Control Group						
	Positive			Negative				Positive			Negative			
	Cases	Subject Number	Log μμl Per Day	Cases	Subject Number	Log μμl Per Day	Total Cases	Cases	Subject Number	Log μμl Per Day	Cases	Subject Number	Log μμl Per Day	Total Cases	Total
Subject Having Significant Slope of Threshold at 33.5 Minutes During Six Weeks[2]	1	D60	.016	1	D15	.007	—	1	D54	.007	1	D74	.019	—	—
	1	D46	.008	1	D17	.010	—	1	D91	.014	1	D86	.017	—	—
	1	D82	.006	1	D93	.005	—	1	D25	.002	1	D52	.003	—	—
	1	D84	.004	1	D87	.005	—	1	D80	.005	1	D73	.003	—	—
	1	D108	.006	—	—	—	—	—	—	—	—	—	—	—	—
Total	5			4			9	4			4			8	17
Subjects Having Slope of Threshold at 33.5 Minutes Not Different from Zero[3]							21							12	33
Total							30							20	50

[1] $b = \frac{Sxy}{Sx^2}$, where x represents deviations from the mean observation day (X); y, deviations from the mean threshold at 33.5 minutes (Y) during six weeks; and the summations (S) extend over 8 points.

[2] $p. \leq .05$ for Student's "t" obtained by dividing the individual standard error of estimate by the standard error of the individual slope.

[3] $p. > .05$ by the same criterion.

therapy group who had slopes for threshold differing significantly from zero, four had negative values, indicating a fall in threshold during the study and five had positive slopes, corresponding to a rise in threshold. Similarly in the control group, eight subjects had significant slopes of which half were negative and half positive.

Continued observations on many of the subjects indicated that the similarity of consistent changes in threshold in the therapy and control groups was probably not a result of the moderate level at which therapy was administered, nor due to the short period. After the close of the present study twenty-one subjects of the therapy group and nineteen control subjects were retested from time to time for several months. Seventy-five per cent of these subjects were under observation for at least six months and the remainder for three, four, or five months. The amount of therapy was increased from 10,000 International units of vitamin A daily to 100,000 International units per day. Despite the more extended period and higher level of therapy the changes in threshold at 33.5 minutes were essentially alike in therapy and control groups and they resembled the pattern described in Table 1.

The conclusion appears to be justified, therefore, that any benefits received by subjects of the therapy group through the supplements of vitamin A were not reflected in their visual thresholds late in adaptation. With respect to change in threshold at 33.5 minutes the therapy and control subjects can be regarded as a homogeneous group, in which during six weeks significant rise in threshold level occurred in approximately 18 per cent of the cases and in the same period significant fall in a nearly equal proportion, 16 per cent. Furthermore, during the six weeks thirty-three subjects, or 66 per cent of the total group, were characterized by variations of threshold at 33.5 minutes which were in no part accounted for by a linear trend.

Age Differences. In the group of fifty subjects, whose individual slopes are summarized in Table 1, the mean threshold at 33.5 min-

utes for subjects less than 40 years of age was lower than the mean for subjects 40 years of age or older. In Table 2, mean threshold at 33.5 minutes is shown for subjects in these broad age groups. The variability of threshold in each age group is also given in this table. Mean threshold in the younger age group was 2.698 log $\mu\mu l$ and in the older group 3.010 log $\mu\mu l$. The difference, .312 log $\mu\mu l$, was much larger than could be explained by expected variation due to sampling alone.

The variability between different subjects in the older age group was greater. However, the coefficients of variation in the two age groups were approximately the same.

Variation of Visual Threshold. The two dark adaptation curves resulting from duplicate tests of the same subject were not identical. Similarly, serial adaptation curves for a single individual obtained by periodic testing at two-week intervals differed considerably from

Table 2. Mean and standard deviation of threshold at 33.5 minutes during dark adaptation for subjects who were less than 40 years of age and for subjects 40 years of age or older.

Age Groups	Number of Cases[1]	Number of Observations	Threshold at 33.5 Minutes	
			Mean[2] Log $\mu\mu l$	Standard Deviation[3] for Different Subjects Log $\mu\mu l$
Subjects Less Than 40 Years	31	248	2.698	±.250
Subjects 40 Years or Older	19	152	3.010	±.328
Total	50	400	2.817	±.281[4]

[1] Each of three technicians tested approximately one-third of the subjects in each age group.

[2] SV/N, where V is the threshold (33.5 minutes) in any test at any period in each age group, and N is the number of observations in the age group.

[3] $\sqrt{\Sigma\, Sy^2/(n\text{-}Kk)}$, where y is the deviation of the mean of duplicate thresholds (33.5 minutes) of any subject in a technician-period grouping from the mean threshold in that grouping; y^2 is summed (S) within each technician-period grouping; Sy^2 is summed (Σ) over all technician-period groupings; n is the number of subjects; K, the number of examination periods; and k, the number of technicians. The standard deviation between different subjects was computed within technician groups since thresholds measured by different technicians are not directly comparable (18, 22). The subjects were grouped according to examination period to control also the individual variation of threshold between periods.

[4] This variability of threshold between different subjects is slightly larger than that given in Table 6, item 2, as it includes the error of measurement whereas the variability in Table 6 does not. The value in Table 6 is a better estimate of the variability between different subjects.

one another and indicated temporal changes in the threshold of the individual. An attempt was made to measure each of these types of variation in order to answer the questions: 1, How reliable is a single threshold observation at a given dark time as an index of the individual's threshold at that time in the particular test, and 2, how reliably will a threshold observation in a single test indicate the characteristic threshold of the individual during a six-week period, at the dark time specified? Reliability in the first question depends, of course, upon the immediate error of measurement. The differences between thresholds at each dark time in the duplicate tests provided a means of estimating immediate error. The reliability referred to in the second question is limited by error and also by the amount of temporal deviation of threshold of the individual from his characteristic (mean) level during the limited period. Temporal variation of the individual's threshold at 33.5 minutes was estimated from observations in repeated tests of the same subject at successive examination periods.

1. *Immediate error of measurement throughout adaptation and the effects of experience:* The immediate error described here was derived on differences for all subjects tested in the same period. At each period differences were computed at each of twelve observation intervals (dark times) by subtracting the measurement in the second test of a pair of duplicates from that in the first test. At successive examination periods the differences at a given dark time reflected the response of subjects as their experience with the test increased. In general, subjects tested in period I were inexperienced, subjects in period II had experienced not more than two tests, those in period III not more than four tests, and those in period IV not more than six tests. In the course of repeated testing of the same individuals the technicians may also have become increasingly aware of the characteristic behavior of particular subjects. Significant change in the technician's understanding of the test procedure and facility in conducting the test probably did not occur, inasmuch

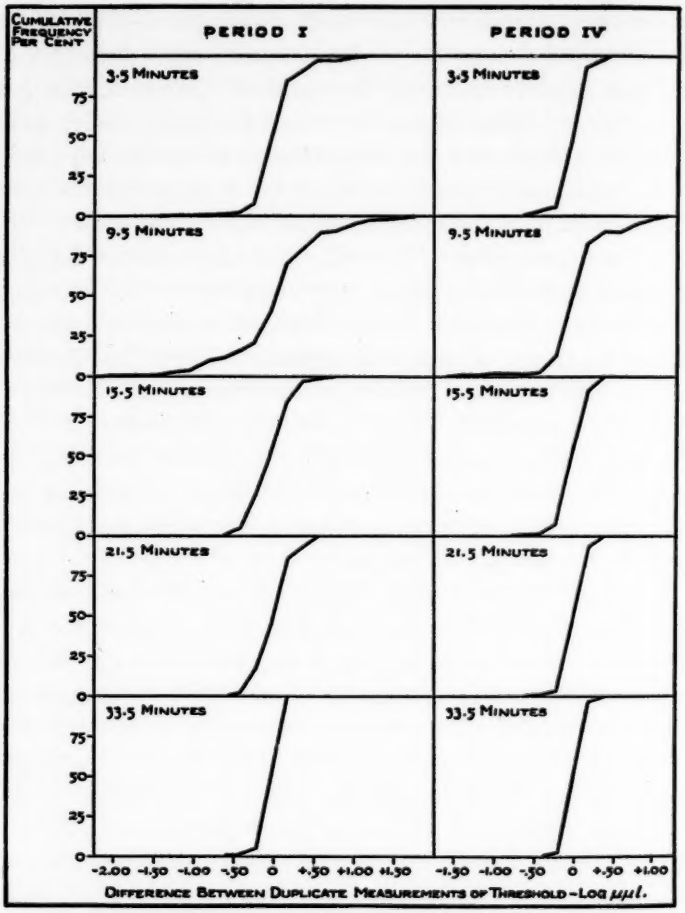

Fig. 1. Cumulative frequency distributions of differences between duplicate measurements of threshold during dark adaptation at each of five dark times for eighty-three inexperienced subjects in examination period I and sixty-nine of the same subjects when experienced in period IV.

as each of the technicians had previously tested several hundred subjects.

Typical distributions of differences between duplicate measure-

ments are illustrated in Figure 1 for eighty-three inexperienced subjects tested in period I and for sixty-nine of the same subjects, tested in period IV. In the latter period 72 per cent of the sixty-nine cases had been tested previously six times and the remainder four times. The curve in each panel of this figure was obtained by arranging the differences in a cumulative frequency distribution. Cumulative frequency at each interval of difference was then expressed in per cent of the total frequency and plotted against the scale of differences, which increased from negative values through zero to positive values of the differences. Distributions are shown at five of the twelve observation intervals during adaptation. At other intervals and also at periods II and III the distributions were similar in type to those illustrated here.

Several general observations can be made on the basis of Figure 1. 1. Positive and negative differences occurred with about equal frequency and the differences showed a tendency to center about zero. 2. In the first examination period the total range of the distribution of differences declined as adaptation proceeded from 0.5 to 33.5 minutes in the dark. 3. After the subjects had become experienced in the fourth examination period a similar but less marked decline in the range was found. 4. At each dark time before 33.5 minutes the total range of the distribution for experienced subjects in period IV was smaller than that for inexperienced subjects in period I.

The mean difference between duplicate measurements of threshold and the standard deviation of this difference for different subjects are shown in Table 3 at each examination period according to time in the dark. The subjects are those whose differences were illustrated in Figure 1. It will be seen in this table that the mean differences for inexperienced subjects (period I), at the beginning and also late in adaptation were significantly different from zero. After the first examination period the mean difference at all dark times showed only insignificant fluctuations from zero. The mean differences showed, therefore, that the mean error of measurement

Dark Time (Minimum)	Difference Between Duplicate Measurements of Threshold											
	Examination Period											
	I			II			III			IV		
	Number of Cases	Mean Log μμl	Standard Deviation Log μμl	Number of Cases	Mean Log μμl	Standard Deviation Log μμl	Number of Cases	Mean Log μμl	Standard Deviation Log μμl	Number of Cases	Mean Log μμl	Standard Deviation Log μμl
0.5	83	+.063[1]	±.266	75	+.014	±.172	66	+.016	±.126	69	−.009	±.135
3.5	83	+.032	±.277	75	+.004	±.200	66	−.002	±.307	69	+.010	±.141
6.5	83	+.014	±.490	75	+.070	±.435	66	+.054	±.430	69	+.022	±.331
9.5	83	+.022	±.564	75	+.063	±.501	66	+.090	±.504	69	−.011	±.382
12.5	83	+.014	±.316	75	−.013	±.343	66	+.053	±.297	69	−.028	±.249
15.5	83	−.022	±.228	75	−.009	±.285	66	+.023	±.217	69	−.007	±.162
18.5	83	+.010	±.216	75	−.006	±.241	66	+.025	±.176	69	+.002	±.156
21.5	83	+.006	±.207	75	+.022	±.182	66	+.004	±.177	69	+.003	±.146
24.5	83	−.031[1]	±.139	75	+.012	±.108	66	+.016	±.157	69	+.021	±.122
27.5	82	−.035[1]	±.121	75	.000	±.113	66	+.007	±.124	69	.000	±.097
30.5	83	−.021[1]	±.087	75	+.002	±.112	66	+.014	±.121	69	−.005	±.100
33.5	82	−.026[1]	±.091	74	−.008	±.109	66	+.007	±.115	67	−.008	±.101

[1] $.05 > p > .01$; for "t", where the value of "t" is the mean difference divided by the standard error of the difference.

Table 3. The mean difference between duplicate measurements of threshold during dark adaptation, and the standard deviation of the difference at each of four examination periods, according to dark time.

was zero, except for inexperienced subjects at .5 minute and 24.5-33.5 minutes dark time. The measurements of inexperienced subjects in other observation intervals and of experienced subjects throughout adaptation were unbiased.

The variability of the error of measurement for experienced and inexperienced subjects is shown in Table 4 according to examination period and dark time. Each value in Table 4 is the standard deviation of the error of measurement derived from the corresponding standard deviation of the differences between duplicate measurements in Table 3. It will be seen that regardless of experience the variability of the error of measurement is largest at 9.5 minutes dark time and smaller after 21.5 minutes than at any other time. The effect of increased experience upon error is clearly illustrated in the smaller variability of error in period IV as compared with period I at each dark time before 24.5 minutes. At 24.5 minutes and later in adaptation, error was unaffected by the extent of experience.

Dark Time	Standard Deviation of Error of Measurement (s_ϵ)			
	Examination Period			
	I Inexperienced Subjects	II Subjects Tested Previously not More Than Twice	III Subjects Tested Previously not More Than Four Times	IV Subjects Tested Previously not More Than Six Times
(Minutes)	Log $\mu\mu l$	Log $\mu\mu l$	Log $\mu\mu l$	Log $\mu\mu l$
0.5	±.188	±.122	±.089	±.095
3.5	±.196	±.141	±.217	±.100
6.5	±.346	±.308	±.304	±.234
9.5	±.399	±.354	±.356	±.270
12.5	±.223	±.243	±.210	±.176
15.5	±.161	±.201	±.153	±.115
18.5	±.153	±.170	±.124	±.110
21.5	±.146	±.129	±.125	±.103
24.5	±.098	±.076	±.111	±.086
27.5	± 086	±.080	±.088	±.068
30.5	±.062	±.079	±.086	±.071
33.5	±.064	±.077	±.081	±.071

[1] Standard deviation of the difference between duplicate measurements of threshold divided by $\sqrt{2}$.

Table 4. Variability of the error of measurement[1] of threshold during dark adaptation of any subject at each of the four examination periods according to dark time.

It may be assumed that twice the value of the standard deviation of the error of measurement will include approximately 95 per cent of the errors liable to occur in connection with single measurements of any subject.[3] These limits ($\pm 2S_\epsilon$) define in a uniform way the error component of unreliability of single measurements of threshold at different dark times and following different degrees of experience. For example, the observed threshold of an inexperienced subject (period I) at 6.5 minutes has an error of measurement which will probably not exceed ±.692 log $\mu\mu l$ more than five times out of one hundred.[4] Similarly, for inexperienced subjects at 15.5 minutes the odds are the same that the error of measurement will not exceed ±.322 log $\mu\mu l$. For measurements of experienced subjects,

[3] The form of the distributions shown in Figure 1 appear to justify this assumption with the possible exception of the distribution at 9.5 minutes.

[4] Assuming that the variance of error for other samples of the same size will not differ greatly from that of the present sample.

tested previously not more than six times, the corresponding limits of error at the same dark times are ±.468, ±.230 log $\mu\mu l$, respectively.

The only evident learning effect in the present study was an increase in the immediate reliability of single observations before 24.5 minutes. Experience did not alter the level of the individual's threshold consistently in one direction. As indicated by the findings presented in Table 1 linear trend of threshold at 33.5 minutes was absent in the majority of cases and the significant trends were as frequently in the direction of elevated as of lowered thresholds. A graphical analysis at 30.5 and 27.5 minutes gave similar results. Before 27.5 minutes the same tendencies prevailed. It will be seen in Table 5 and the corresponding Figure 2 that mean thresholds in periods I and IV did not differ significantly at any dark time. Thus after having taken six tests (period IV), the mean threshold of the

Table 5. Mean threshold during dark adaptation in examination periods I and IV for fifty-three subjects who were tested at each of four periods, according to dark time.

Time in the Dark (Minutes)	Number of Cases	Number of Observations	Mean Threshold[1]	
			Period	
			I Log $\mu\mu l$	IV Log $\mu\mu l$
.5	53	106	6.525	6.457
3.5	53	106	5.827	5.781
6.5	53	106	5.271	5.314
9.5	53	106	4.383	4.368
12.5	53	106	3.766	3.779
15.5	53	106	3.433	3.446
18.5	53	106	3.191	3.224
21.5	53	106	3.021	3.024
24.5	53	106	2.886	2.911
27.5	53	106	2.809[2]	2.832
30.5	53	106	2.773	2.807
33.5	52	104	2.761	2.804[3]

[1] Mean threshold in the first and in the second test for subjects tested in the period at the dark time specified.
[2] n = 104 observations for 52 subjects.
[3] n = 102 observations for 51 subjects.

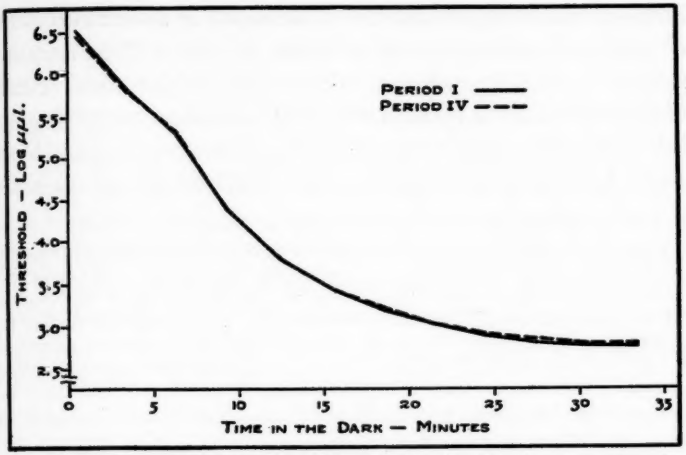

Fig. 2. Mean threshold during dark adaptation for fifty-three inexperienced subjects (period I) and for the same subjects after having taken six tests (period IV).

fifty-three subjects was the same at each dark time as in period I, when they were without previous experience.

2. *Temporal variability of threshold and immediate error of*

Table 6. Variability of threshold at 33.5 minutes during dark adaptation for fifty subjects tested in duplicate at each of four examination periods, according to source of variation.

Source of Variability	Standard Deviation of Threshold ($\bar{\sigma}$) Log $\mu\mu l$	Proportion of Variance ($\bar{\sigma}^2$) Per Cent
1. Total, $\bar{\sigma}_{X_{ijk}}$ (400 Observations).	±.316	100.0
2. Different Subjects, $\bar{\sigma}_y$ (50 Individuals).	±.253	64.0
3. Temporal Changes of the Individual's Threshold during Six Weeks, $\bar{\sigma}_t$ (Four Examination Periods).	±.173	30.0
4. Immediate Error of Measurement, $\bar{\sigma}_\epsilon$ (200 Pairs of Duplicate Observations).	±.077	6.0
5. Immediate Error of Measurement and Temporal Changes ($\sqrt{\bar{\sigma}_t^2 + \bar{\sigma}_\epsilon^2}$).	±.189	36.0

measurement at 33.5 minutes: The variability of 400 measurements of threshold at 33.5 minutes is shown in Table 6.[5] The measurements were duplicate observations on fifty subjects tested at each of four examination periods. The total variability, $\pm .316$ log $\mu\mu l$ was a composite of variations arising from error, and temporal deviations from mean of the individual's threshold during the study

[5] The standard deviations (σ) shown in Table 6 were obtained in an analysis in which the assumptions and procedures were as follows: Any threshold (Y) was assumed to be the sum of (1) an error component, ϵ, (2) a temporal fluctuation, t, and (3) the individual's characteristic threshold value, y. According to this view $Y_{ijk} = \epsilon_{ijk} + t_{ij} + y_i$, where i = the individual, j = examination period, and k = a single observation in any period. It was assumed also that: ϵ_{ijk}, t_{ij}, and y_i are mutually uncorrelated; $Et_{ij} = E\epsilon_{ijk} = 0$ for all i, j, k, and $E\,y = m$ for all i, where the symbol E represents the expected or mean value of the quantity immediately following it. As a rough approximation, it was assumed furthermore that $\sigma^2_{t_{ij}} = \sigma^2_t$ for all i and j, and $\sigma^2_{\epsilon_{ijk}} = \sigma^2_\epsilon$ for all i, j, and k. The symbol σ^2_y will be used to represent the variance of y_i.

The total sum of squares is given by $\sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{q}(Y_{ijk} - \overline{Y})^2$, where $\overline{Y}$ is the general mean of all observations, n = the number of individuals, p = the number of periods, and q = the number of repeated tests of a subject in a given period. This sum of squares is the sum of three parts:

(a) $\sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{q}(Y_{ijk} - \overline{Y}_{ij.})^2$, in which $\overline{Y}_{ij.}$ is the sum of any pair of duplicate observations;

(b) $q\sum_{i=1}^{n}\sum_{j=1}^{p}(\overline{Y}_{ij.} - \overline{Y}_{i..})^2$, in which $\overline{Y}_{i..}$ is the mean for any individual of all observations at all periods; and finally

(c) $pq\sum_{i=1}^{n}(\overline{Y}_{i..} - \overline{Y})^2$.

The expected values for the sums of squares indicated under a, b, c are:

(1) $E\sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{q}(Y_{ijk} - \overline{Y}_{ij.})^2 = np(q-1)\,\sigma^2_\epsilon$

(2) $E\,q\sum_{i=1}^{n}\sum_{j=1}^{p}(\overline{Y}_{ij.} - \overline{Y}_{i..})^2 = nq(p-1)\,\sigma^2_\epsilon + nq^2(p-1)\,\sigma^2_t$

(3) $Epq\sum_{i=1}^{n}(\overline{Y}_{i..} - \overline{Y})^2 = (n-1)\,\sigma^2_\epsilon + q(n-1)\,\sigma^2_t + pq\,(n-1)\,\sigma^2_y$

These expressions show that: the expected value of $\sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{q}(Y_{ijk} - \overline{Y}_{ij.})^2$ is a function of the variance of error (σ^2_ϵ), the expected value of $q\sum_{i=1}^{n}\sum_{j=1}^{p}(\overline{Y}_{ij.} - \overline{Y}_{i..})^2$ is a function of the variance of error (σ^2_ϵ) and of temporal fluctuations (σ^2_t), and the expected value of $pq\sum_{i=1}^{n}(\overline{Y}_{i..} - \overline{Y})^2$ is a function of σ^2_ϵ, σ^2_t, and the variance of the characteristic thresholds of the different subjects (σ^2_y).

(Continued on page 155)

and differences in the characteristic thresholds of different subjects. Sixty-four per cent of the total variance represented differences between the subjects. This variability, $\pm.253$ log $\mu\mu l$, reflects age effects upon visual function, one aspect of which was noted here, and, presumably, differences with respect to many other factors affecting threshold, which are relatively stable over a period of time, for example: intelligence and educational level (51); individual functional patterns of the sympathico-adrenal system (30); individual efficiency in the utilization of any of the nutrients essential to retinal and cerebral metabolism, relative to the individual's characteristic supply of these nutrients; and pupil size in the light and in the dark (35).

Temporal deviations of the individual's threshold and error accounted for 36 per cent of the total variance and of this proportion the greater part, 30 per cent, arose from temporal variability. The individual temporal variability, $\pm.173$ log $\mu\mu l$, was significantly greater than the immediate error of measurement, $\pm.077$ log $\mu\mu l$.

Hence an unbiased estimate for σ^2_ϵ is given by $\sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{q}(Y_{ijk}-\bar{Y}_{ij.})^2$; an unbiased estimate of σ^2_t is given by $\frac{\sum_{i=1}^{n}\sum_{j=1}^{p}(\bar{Y}_{ij.}-\bar{Y}_{i..})^2}{nq(p-1)}-\frac{\sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{q}(Y_{ijk}-\bar{Y}_{ij.})^2}{npq(q-1)}$; and an unbiased estimate of σ^2_y is given by $\frac{\sum_{i=1}^{n}(\bar{Y}_{i..}-\bar{Y})^2}{n-1}-\frac{\sum_{i=1}^{n}\sum_{j=1}^{p}(\bar{Y}_{ij.}-\bar{Y}_{i..})^2}{npq(p-1)}$

The values in Table 6 of σ_y (item 2), σ_t (item 3) and σ_ϵ (item 4) are square roots of weighted mean variances ($\bar{\sigma}^2_y$, $\bar{\sigma}^2_t$, $\bar{\sigma}^2_\epsilon$) for the three technician-groupings.

The variances of σ^2_y, σ^2_t, and σ^2_ϵ were computed initially within technician-groupings since different technicians do not obtain exactly the same threshold in testing the same subject, other conditions being equal. Accordingly, the variance, σ^2_y, if computed without regard to technicians, would probably be somewhat larger than the value given in Table 6, and unless the technicians were equally consistent in measurement procedure over a period of time σ^2_t would also be affected. In the case of σ^2_ϵ, data of the present study indicated that this quantity at 33.5 minutes was the same for all technicians.

The analysis within technician-groupings showed that the proportions of $\sigma^2_{Y_{ijk}}$ contributed by σ^2_ϵ, σ^2_t, and σ^2_y respectively, varied somewhat in the 3 technician-groupings, but in all such groupings σ^2_y accounted for the largest proportion of the variability, σ^2_t was next in importance, and σ^2_ϵ was the smallest component, the order of importance for $\bar{\sigma}_y$, $\bar{\sigma}_t$, and $\bar{\sigma}_\epsilon$ shown in Table 6. Under these circumstances the use of weighted mean variances for the different technicians was considered justified.

The greater variability of serial measurements of the individual, as compared with duplicate measurements, might be expected, since in duplicate tests within approximately one and a half hours the physiological state of the subject is relatively stable, whereas in serial testing the measurements may vary as a result of temporary fluctuation of factors affecting threshold. The latter fluctuations may be due to differences in certain aspects of test conditions at different examination periods, such as unequal ventilation of the dark room or variations in the amount of bright sunlight to which the subject is exposed on different examination days. They may also include, for example, variations in the concentration of vitamin A in the blood arising from dietary changes or for other reasons (49, 50, 33) and transitory changes in level of blood sugar (29).

In practice, any threshold measurement of the individual in a particular test reflects immediate error and the factors of temporal variation jointly. The standard deviation for the combined variability due to both sources was ±.189 log $\mu\mu l$. This result indicates that threshold of any subject in a particular test deviates very frequently from the individual's characteristic mean level of threshold during a limited period (six weeks) to the extent of ±.189 log $\mu\mu l$, higher or lower. Since temporal variations as well as immediate error appeared to be normally distributed it is likely that the limits ±.189 log $\mu\mu l$ would account for approximately 68 per cent of the deviations from characteristic individual level. If no more than 5 per cent of the deviations are disregarded, ±.378 log $\mu\mu l$ would appear to be required.

From the above considerations it follows that in the population group represented here a single threshold measurement at 33.5 minutes is commonly unreliable as an index of the individual's characteristic mean level within a limited period to the extent of ±.189 log $\mu\mu l$ and, less frequently, ±.378 log $\mu\mu l$.

The unreliability of single threshold measurements, ±.378 log $\mu\mu l$, can be used to indicate roughly the amount of change in

threshold of the individual from one examination period to another which is to be expected in view of temporal variation and error of measurement and, therefore, need not be regarded as evidence of a significant shift in the characteristic threshold level of the individual. In single tests two weeks apart a change in threshold at 33.5 minutes, which is less than $\pm.523$ log $\mu\mu l$[*] is of doubtful significance and only changes as great as or exceeding $\pm.688$[†] log $\mu\mu l$ can be considered highly significant.

Comment

1. Serial measurements of dark adaptation failed to indicate that subjects who received vitamin A therapy were benefited. Assuming that visual threshold, measured at 33.5 minutes in the dark, is closely related to the intake of vitamin A, this result might be expected if the body reserves of vitamin A were already adequate. The latter presumption of adequate reserves of this vitamin does not appear to be justified, in as much as eye examinations in the gross and also by biomicroscope showed conjunctival lesions in 72 per cent of the eighty-three subjects in the present study and regression of these lesions in cases receiving vitamin A therapy was reported (24). It seems highly probable, therefore, that the group concerned here contained a fairly large number of cases of avitaminosis A of some degree. The latter view receives some support also from the fact that the subjects were drawn from an unmistakably underprivileged group in which average annual family income was approximately $750. At this income level the food expenditure of 50 per cent of the families concerned might be expected to be insufficient to secure nutritionally adequate diets (47). Great economy in expenditures combined with scientific planning of the diets would alter this expectation. In the group studied it is doubtful that more than a few individuals possessed requisite training and ex-

[*] $\pm$ "t".05 S $\bar{d}$, where "t".05 = 1.960 and S $\bar{d} = \sqrt{2(\bar{\sigma}_i^2 + \bar{\sigma}_e^2)}$.

[†] $\pm$ "t".01 S $\bar{d}$; "t".01 = 2.576.

perience to secure regularly well-balanced diets under adverse economic circumstances.

The similarity of threshold changes at 33.5 minutes in the therapy and control groups suggests a lack of relationship between threshold measurements at this dark time and vitamin A in the diet. This finding is at variance with the evidence from a number of depletion experiments with human subjects in which a correlation between threshold and intake of vitamin A was shown by the rise of threshold, which accompanied a greatly reduced supply of this nutrient in the diet (20, 6, 44, 15, 16). Blanchard and Harper also found, early in adaptation, an increase in "adaptation time" among students whose intake of vitamin A was reduced to a low level for 42-45 days (4). Furthermore, highly probable cases of vitamin A deficiency, diagnosed on the basis of low vitamin A content of the serum as well as relatively high threshold late in adaptation, have shown marked improvement in adaptation under vitamin A therapy (26). Similar cases of high threshold late in adaptation due to vitamin A deficiency and responding to vitamin A therapy, were reported recently by Rajagopal (36), Yudkin (50), and Campbell (8). On the other hand the lack of evidence of relationship between threshold as measured and intake of vitamin A in the present study is in line with the results of experiments in which attempts to produce nightblindness in human subjects by diets low in vitamin A were unsuccessful (39, 19, 45, 11). In view of the latter reports and results in the present study, it appears that the correlation of threshold and intake of vitamin A is less direct than indicated by the findings in the first group of experimental and clinical investigations cited here.

Studies of the correlations between threshold measurements and vitamin A in the blood and in the diet also have not revealed a uniformly high degree of relationship among the three variables. Three groups of investigators (41, 2, 9) found no correlation between threshold and vitamin A in the blood, but the intake of

vitamin A affected the concentration of vitamin A in the blood. The biophotometer was used to measure threshold early in adaptation. The instrument, customary procedure and results obtained have been critically reviewed elsewhere (26, 32, 38). Similar results were obtained by Yarbrough and Dann (48) for the nearly dark adapted threshold measured with the adaptometer and the concentration of vitamin A in plasma determined from non-fasting blood samples of 154 subjects in a southern rural mill town. The correlation between the threshold and vitamin A in plasma was +.131 and not significant. Josephs, Baber, and Conn (21) in a study of 147 persons, mostly children, found an increase in mean "recognition time" (after about 4 minutes of dark adaptation) associated with a mean decrease in the amount of vitamin A in the serum when subgroupings of subjects at progressively poorer dietary and socio-economic levels were compared. When paired values of "recognition time" and vitamin A in plasma for different individuals were correlated little relationship between the variables was found. Bodansky, Lewis, and Haig (5, 25) observed a significant mean difference in the plasma vitamin A concentration of forty-six infants, 3 weeks to 6 months of age, receiving 1,200-1,500 units of vitamin A daily and forty-seven infants of the same age on the same diet but supplemented with 17,000 units of vitamin A daily. A corresponding difference in mean thresholds late in adaptation was not present. When vitamin A in the diet was reduced to 335 units daily, a decrease in plasma concentration of vitamin A followed without the appearance of abnormal adaptation. The latter appeared, however, when vitamin A was entirely withheld from the diet and the plasma vitamin A concentrations fell below 45 units of 100 cc. of plasma (seven cases). Administration of small amounts of vitamin A (150 units daily for thirty days) returned the threshold to normal but were insufficient to increase the plasma concentrations to normal level. The results showed that the concentration of vitamin A in plasma in infants is

more closely related to vitamin A deficiency than threshold late in adaptation. Lindquist (26) found that clinical patients (fifty adults less than 40 years of age) with low concentrations of vitamin A in serum (less than 100 I.U. per 100 cm.) had a higher mean threshold (late in adaptation) than similar subjects (175 cases) whose vitamin A content of serum was 100 I.U. per cm. or more. For subjects more than 40 years of age and with the serum concentration of vitamin A less than 100 I.U. per 100 cm. (thirty-seven cases) a significant correlation (.29) between threshold and vitamin A concentration of serum was observed, whereas none was found when the vitamin A content of serum was 100 I.U. per cm. or greater. In a study of 214 healthy persons and thirty-one patients, Saksela (37) reported normal adaptation in 85.2 per cent of the subjects whose vitamin A content of the serum was 226 I.U. per cc. or greater, as compared with 18 per cent in the group in which the vitamin A of serum was 120 I.U. per cc. or less. Twenty-six individuals observed by Pett and Le Page (34) showed a relationship regarded as significant between "regeneration time" (early in adaptation) and the vitamin A concentration of plasma.

2. The conflicting appraisals of the degree of relationship between threshold measurements, intake of vitamin A and the vitamin A content of the blood, raise the question whether, frequently, the actual relationships may not be obscured either by inherent inaccuracies of the procedures employed or by lack of control over factors and circumstances which greatly increase the variability of the observations. It may be asked in the present study, for example, whether a preadaptation in the neighborhood of 1,004 millilamberts provided a searching appraisal of individual capacity with respect to dark adaptation. In studies of nutritional nightblindness in rats, a prolonged preadaptation at relatively high brightness appeared to be an important factor in the experimental production of impaired dark adaptation (13). Dark adaptation following preadaptation at relatively low brightness may be well within the range of successful

performance for even moderately impaired capacity whereas recovery from more intense light exposure may tend to differentiate more clearly, excellent, average, and poor capacity. It should be noted also that the test, as given, examined but a small field of the retina.

3. The age difference noted was similar to those described elsewhere. In a study employing the adaptometer a slight tendency of the nearly dark adapted threshold to be distributed toward higher values than were common in younger age groups was evident among persons 40 to 65 years of age (15). Basu and De, using an instrument similar to the adaptometer, reported thresholds after 15-30 minutes dark adaptation in a group of 341 school children which were on the average lower than those obtained for 161 adult employees (1). Higher and more variable threshold of the nearly dark adapted eye in persons over 40 years of age was found in examinations with the Gullstrand photometer (26). With another apparatus and procedure, "light minimum" of persons over 35 years of age was found to be higher than that of younger subjects (12). A diminished power of visual distinction among older persons has also been observed (7). Wittkower and Rodger (51) and also Stewart (43) reported a correlation of adaptation ability with age. Some part of this correlation is apparently due to a decrease in the size of the pupil in the dark in older persons (35).

4. The variations of threshold measurements described in the present paper differ in two respects from those reported heretofore for the adaptometer or a similar instrument. In the first place, the errors of single measurements are larger in size than those reported either for adults at a nonrelief level of income or for practiced laboratory subjects tested by the same or a closely similar procedure (44, 15, 46, 27). For the types of subjects mentioned the "generally expected" error at one sitting is .10 log units. Yudkin gives the figure, .10 log unit, for variation of the individual's threshold within a few days (50). In the present study .10 log unit ($0 \pm .05$ log $\mu\mu l$)

accounted for only about 30 per cent of the errors of single measurements late in adaptation. The limits including about 95 per cent of the errors were ±.151 log $\mu\mu l$ or an interval of .30 log $\mu\mu l$ for the nearly dark adapted eye. Second, the error of single measurements was not constant throughout adaptation. Measurements were least reliable in the neighborhood of 6.5-12.5 minutes and had the smallest error at 30.5-33.5 minutes dark time. In connection with the measurement of the limit of the "cone" threshold and the "transition" time, Jung and Greenberg have also reported larger observational errors early in adaptation than for the nearly dark adapted eye (22).

Inasmuch as the fluctuation in error during adaptation decreased significantly as the subjects became more experienced and the technician better acquainted with individual subjects, it is clear that a definite learning factor was operative during the first 20-25 minutes of adaptation. The pattern of error indicates that "poor" subjects are commonly met in routine field testing. This appraisal of error in routine testing is consistent with the comments of McDonald and Adler with regard to allowances which must be made in the interpretation of threshold measurements of clinical and ward patients (28).

5. A criterion of vitamin A deficiency, employing measurement of the threshold of the nearly dark adapted eye has recently been proposed (42), and commented upon (48, 50). The procedure of measurement was similar to the one used here. According to this criterion a decrease in threshold of the dark adapted eye of at least .3 log unit accompanying vitamin A therapy or eventual lowering by this amount when therapy is continued at least two weeks may be regarded as evidence that the threshold is "vitamin A labile." In the population group represented here, a .3 log unit change in threshold of the nearly dark adapted eye might occur more frequently than 5 times out of 100 without therapy, due simply to temporal and error variations of the individual's threshold. A just

significant difference between thresholds at two examinations, two weeks apart, was considerably larger than .3 log units, *i.e.*, ±.523 log units and the highly significant change was more than twice as great, ±.688 log units. The difference .5 log unit was previously suggested by Steffens, Bair, and Sheard (40).

6. The large variability of temporal change and error for threshold measured at 33.5 minutes, ±.189 log $\mu\mu l$ (36 per cent of total variance) suggests that distinctions between individuals with respect to characteristic thresholds can not be made with certainty in this and similar population groups when factors associated with temporal variation, particularly, are uncontrolled. For, if different subjects are tested in one examination period, the differences between them will be due to a considerable extent to the fact that they are in different phases or stations of individual temporal change from characteristic level in a limited period. Jung and Greenberg reported a similar expectation for individuals in a more privileged group, *i.e.*, medical and graduate university students (22). Furthermore because of temporal and error variations, different individuals tested in one period can not be ranked accurately in respect to characteristic threshold, relative to narrow standards of normal response such as have been employed in many population surveys. A critical review of the latter studies up to 1938 is given by Lindquist (26).

The relatively large temporal and error variations of threshold at 33.5 minutes, together with the fact that threshold level late in adaptation in a particular test is not a good indication of how the individual will respond to vitamin A (16) or other therapy administered subsequently, lead to the conclusion that the incidence of nightblindness in a population group can not be accurately determined by means of a cross-sectional survey of thresholds late in adaptation. Since error increases rapidly at earlier dark times and there is no reason to expect smaller temporal variations before 33.5 minutes, the cross-section survey gives results which are probably undependable throughout adaptation.

If significant regression of the nearly dark adapted threshold in response to vitamin A therapy is used as a criterion of nightblindness due to vitamin A deficiency, individuals must be observed serially over a period of time, a procedure unsuited for practical reasons to the study of a large number of individuals. From the latter and previous considerations it appears that threshold measurements may be useful and practicable for the diagnosis of nightblindness due to vitamin A deficiency in laboratory or clinical or very small scale field investigations but undependable or impractical in studies of a large population group.

Summary

The dark adaptation of fifty under-privileged adults was measured periodically, at intervals of two weeks, during a period of six weeks. Supplements of 10,000 International units of vitamin A were given daily to one-half of the group during the study.

1. The adaptation of individuals receiving therapy was not improved.

2. Experience in taking the adaptation test decreased the error of threshold measurements made before 24.5 minutes in the dark but did not affect error at this dark time or others late in adaptation (27.5, 30.5, 33.5 minutes). Throughout adaptation mean threshold level for the group of subjects was unaltered by experience.

3. Regardless of the amount of experience threshold measurements before 20 minutes in the dark were subject to larger observational errors than those made after 20 minutes of adaptation. At 33.5 minutes the error of measurement was $0 \pm .077$ log $\mu\mu l$. Approximately 95 per cent of the errors were within the limits $\pm .151$ log $\mu\mu l$, or an interval .302 log $\mu\mu l$ in width.

4. Due to error of measurement and individual temporal variation, the threshold of the nearly dark adapted eye (33.5 minutes) deviated in any particular test from the individual's characteristic (mean) threshold during a six-week period. The standard devia-

tion of these variations was ±.189 log $\mu\mu l$, which represented 36 per cent of the total variance of threshold at 33.5 minutes.

5. The variability of thresholds of different individuals at 33.5 minutes was ±.253 log $\mu\mu l$, or 64 per cent of the total variance.

6. In semi-monthly examinations in the population group studied smaller changes in the individual's threshold at 33.5 minutes than ±.523 log $\mu\mu l$ are not significant evidence of change in the individual's characteristic threshold. A change as large or greater than ±.688 log $\mu\mu l$ would appear to be highly significant as such differences are expected in no more than 1 per cent of fluctuations due to temporal variation and error of measurement.

7. If nightblindness due to vitamin A deficiency is to be determined by means of threshold measurements, serial study of the individual is necessary during a period of administered intake of vitamin A, as (a) the temporal and error variations of the individual's threshold are large, (b) the threshold level in a particular test does not indicate the probable response to therapy, and (c) threshold level is affected by many other factors as well as vitamin A.

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MEDICAL EVALUATION OF NUTRITIONAL STATUS

X. SUSCEPTIBILITY TO DENTAL CARIES AND FAMILY INCOME[1]

HENRY KLEIN AND CARROLL E. PALMER, M.D.

OBSERVATIONS (1) on nearly a quarter million white elementary school pupils have indicated that children who live in affluent communities and those who reside in relatively poor urban areas, display, in general, a quite similar tendency to be attacked by dental caries. On the basis of those findings, it would appear reasonable to conclude that the socio-economic status *of a community* has no significant influence on the amount of dental caries experienced by children of school age. The results of the present study support this conclusion. The material to be discussed here reveals that two groups of New York City children —one attending a private, the other a public, school—are attacked by dental caries to about the same degree irrespective of the fact that marked socio-economic differences exist between the two groups.

Persons Examined. The data of the present report consist of dental examinations of the 2,595 New York City high school pupils, shown by age, sex, and income level in Table 1. Of those children, 2,242 were attending a public high school on the lower East Side of Manhattan (Seward Park High School), and 353 were enrolled in a private school in the suburban metropolitan area (Fieldston School).

Many children in both schools were of Jewish parentage. At the

[1] The dental examination findings on which this report is based are part of a larger group of data collected for studies on the Medical Evaluation of Nutritional Status. The studies were undertaken as a cooperative investigation by the Milbank Memorial Fund; the New York City Department of Health; the Division of Public Health Methods of the National Institute of Health, United States Public Health Service; and the Cornell University Medical College, Department of Public Health and Preventive Medicine and Department of Pediatrics.

The cooperating agencies have been assisted in carrying out the investigations by the Work Projects Administration for the City of New York, Official Project No. 65-1-97-21-W.P. 24, "Medical Evaluation of Nutritional Status."

Published papers of the project are listed under Nos. 3 to 10 in the bibliography.

Income Level and Sex	Chronological Age at Last Birthday									
	11	12	13	14	15	16	17	18	19	All Ages
Boys										
High	4	27	38	29	30	28	18	7	1	182
Low	–	4	30	148	274	355	287	103	13	1,214
Girls										
High	5	24	24	24	32	31	28	3	—	171
Low	–	2	29	132	326	322	169	43	5	1,028
Total	9	57	121	333	662	736	502	156	19	2,595

Table 1. New York City school children who received dental examination, distribution by income level of parents, and by sex and age.

public school about 15 per cent of the children were of Italian descent and 75 per cent in all were from families in which both parents were foreign born. Private school children were predominantly of American-born Jewish parentage.

The income levels represented at the two schools were distinctly different. The children attending the private school were from families of high income, whereas the pupils at the public school were from families of which more than 86 per cent had incomes of less than $10 a week per capita and nearly one-third were receiving relief from public or private agencies.

Measurement of Caries Susceptibility. The pupils at the two schools were given complete dental examinations by a dentist using mirror and explorer. The examination results were recorded according to the procedure described in detail in an earlier report (2). A child's susceptibility to dental caries was measured by the number of his permanent teeth showing evidence of caries experience (i.e., the number of decayed, missing, and filled teeth). Any tooth so stigmatized is designated by the symbol "DMF". Comparison is made of the caries tendency of children in the two income groups in terms of the average number of DMF teeth per child and the percentage of children showing specified numbers of DMF teeth.

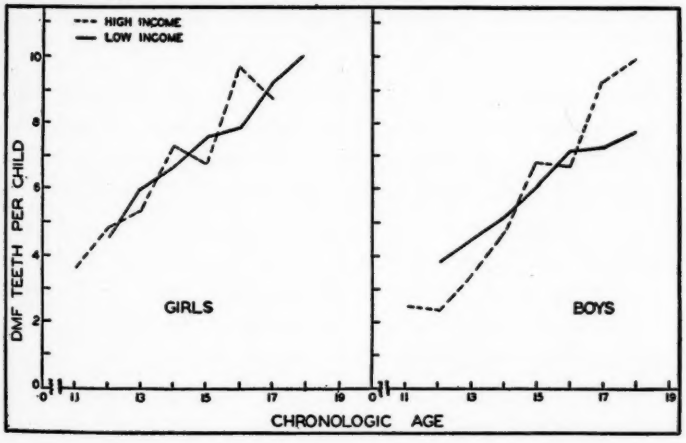
Income Level and Sex	Chronological Age at Last Birthday								
	11	12	13	14	15	16	17	18	19
Boys									
High	2.5	2.4	3.4	4.7	6.9	6.7	9.3	10.0	0
Low	—	3.8	4.5	5.2	6.2	7.2	7.3	7.8	6.4
Girls									
High	3.6	4.8	5.3	7.3	6.8	9.7	8.8	3.7	—
Low	—	4.5	6.0	6.6	7.5	7.8	9.2	10.1	9.6

Table 2. Average number of DMF teeth per child among 2,595 New York City high school children, arranged by income level, sex, and age.

Findings. The average numbers of DMF permanent teeth among children of high and low-income families are shown, by sex and age, in Table 2. The data indicate that children of either income level tend to show, at the same chronological age, about the same average number of DMF teeth. The conclusion is illustrated graphically by Figure 1.

Further substantiation of these findings is provided by the material of Table 3, for which graphic illustration is given in Figures 2 and 3. The percentages of children who have specified numbers

Fig. 1. Number of DMF teeth per child by chronological age; New York City high school pupils arranged by sex and income level.



Number of DMF Teeth	Income Level of Parents			
	Boys		Girls	
	High	Low	High	Low
None	11.5	7.9	8.0	2.3
1	6.1	4.8	0.7	2.6
2	8.6	5.7	4.4	5.2
3	3.9	8.4	3.7	5.2
4	11.7	10.0	12.9	8.1
5	5.7	8.8	3.7	8.0
6	10.6	12.6	12.0	9.5
7	7.5	10.3	6.9	10.5
8	10.6	8.4	9.1	13.2
9	5.7	5.8	7.6	10.7
10	3.2	3.9	8.2	6.7
11	2.1	3.7	6.9	3.9
12	2.0	2.9	2.1	4.4
13	2.1	1.8	2.7	2.5
14	1.7	1.1	2.0	1.9
15	2.5	2.7	1.5	1.7
16	—	0.4	2.7	1.3
17	1.8	0.2	1.6	0.8
18	0.7	0.3	0.7	0.5
19	—	0.2	0.7	0.3
20 or More	2.2	0.7	2.2	0.5
Total	100.0	100.0	100.0	100.0

Table 3. Age-corrected percentages of New York City high school children of each sex having specified numbers of DMF teeth. Data based on 2,354 children aged 13 to 17 years, and arranged by income level.

of DMF permanent teeth are in the main quite similar for both income groups. For example, about 5 per cent of the girls of low-income families and 4 per cent of the girls of high-income families have 2 DMF teeth; 50 per cent of the girls of high, and about the same percentage of girls of low-income level have as many as 7 DMF teeth; and nearly 3 per cent of the girls of the low-income group, and practically the same percentage of those of high income, have as many as 13 DMF teeth. A similar parallelism obtains for both income groups of boys. These several findings indicate rather clearly that family income has no selective effect on the caries susceptibility of the high school children.

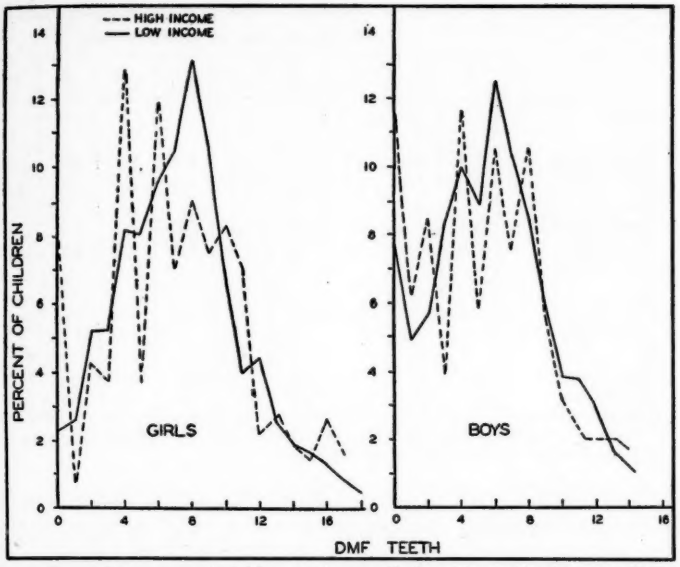
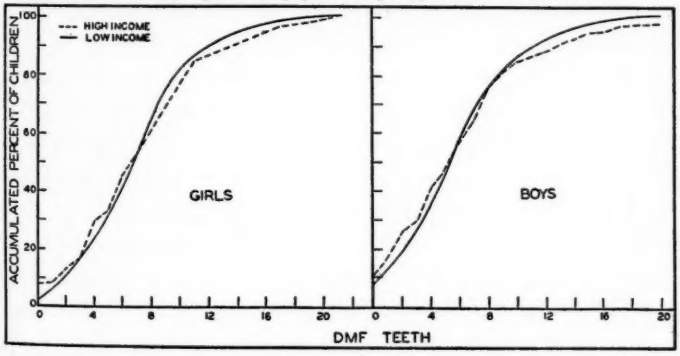


Fig. 2. Percentages of children having specified numbers of DMF teeth; New York City high school pupils arranged by sex and income level.

Comment. It is generally recognized that economic status and health are closely related. It has been clearly shown that persons of

Fig. 3. Accumulated percentages of children having specified numbers of DMF teeth; New York City high school pupils arranged by sex and income level.

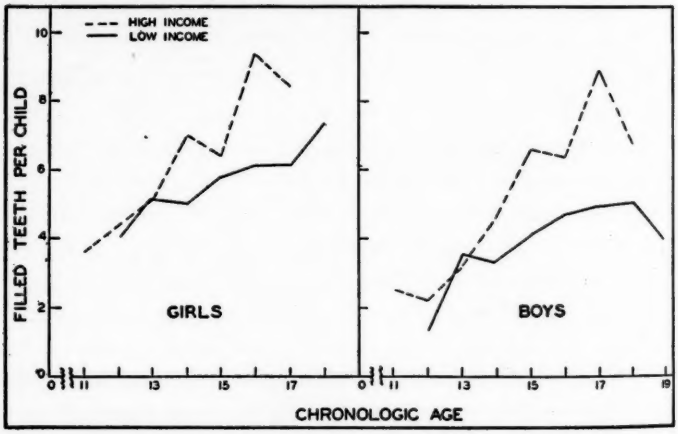


Item	Chronological Age at Last Birthday								
	11	12	13	14	15	16	17	18	19
Filled Teeth									
Boys									
High Income	2.5	2.2	3.2	4.6	6.6	6.4	8.8	6.7	0
Low Income	—	1.3	3.5	3.3	4.1	4.7	4.9	5.0	3.9
Girls									
High Income	3.6	4.4	5.0	7.0	6.4	9.4	8.4	3.3	—
Low Income	—	4.0	5.1	5.0	5.8	6.1	6.1	7.3	9.0
Extracted Teeth									
Boys									
High Income	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0
Low Income	—	0.5	0.3	0.7	0.9	1.1	1.3	1.1	0.9
Girls									
High Income	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.3	—
Low Income	—	0.0	0.4	0.6	0.8	1.0	1.3	2.1	0.6

Table 4. Average number of filled and extracted teeth per child among 2,595 children, arranged by income level, age, and sex.

low-income level have, in general, more illness than individuals in comfortable economic circumstances (11, 12, 13). In recent findings of the National Health Survey (14), it is reported that among persons on relief, the occurrence of nonrespiratory tuberculosis is

Fig. 4. Number of filled teeth per child, by chronological age; New York City high school pupils arranged by sex and income level.



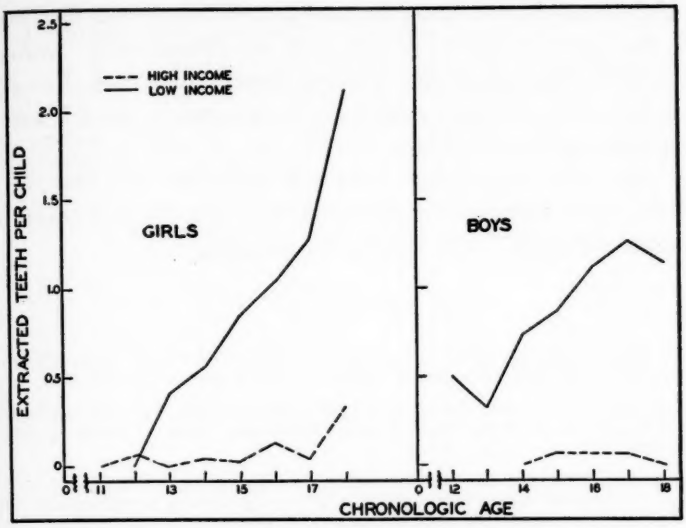


Fig. 5. Number of extracted teeth per child, by chronological age; New York City high school pupils arranged by sex and income level.

eight times as frequent; that of blindness and deafness, five times; orthopedic impairment, three times; nervous and mental diseases, nearly three times; and disabilities due to accident twice as frequent as among persons in families with incomes of $3,000 or more a year.

The findings reported here provide a striking exception to the more or less general rule. The material of the present report reveals that among children from well-to-do, and among those from relatively poor families, there are about equal numbers who have few carious teeth and about as many of the one group as of the other have large numbers of teeth that have been attacked by caries. The children of the families of high income receive diets of better quality and quantity (8, 9). The data in Table 4, shown graphically in Figures 4 and 5, indicate also that the well-to-do children receive more dental care in the form of fillings and they have fewer teeth extracted. In spite of the fact that the environment of the well-to-do

children may be assumed to be more favorable for health than that of the poorer children; yet the clear-cut differences in environmental factors such as diet, housing, dental fillings, and other extrinsic variables seem to produce little difference in the caries susceptibility of the two groups.

These observations taken as a whole lead to the view that caries susceptibility is determined by factors other than the environmental variables associated with socio-economic status.

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MEDICAL EVALUATION OF NUTRITIONAL STATUS[1]

XI. AN ANALYSIS OF SOURCES OF ERRORS IN THE PHOTELOMETRIC MACROMETHOD OF DETERMINING ASCORBIC ACID IN PLASMA

DOROTHY G. WIEHL AND MYRON KANTOROVITZ

DETERMINATION of ascorbic acid in plasma by use of the photoelectric colorimeter, according to the method of Mindlin and Butler (1), has provided a convenient, reliable, and objective procedure. This procedure, reported in 1938, has already been widely adopted. A macromethod and a micromethod were described by Mindlin and Butler, and this has made the procedure available in situations in which only small samples of blood could be obtained. There is little information available to indicate the accuracy of colorimetric determinations of ascorbic acid concentrations derived by either the macro or micromethod. In the study, "Medical Evaluation of Nutritional Status," which is a cooperative investigation by the New York City Department of Health, the United States Public Health Service, the Cornell University Medical College, and the Milbank Memorial Fund, one of the principal objectives has been the appraisal of procedures for determining nutritional status (2). A detailed analysis of the colorimetric determination of ascorbic acid in plasma, therefore, was undertaken.

In the Nutrition Study, the macromethod was used routinely and this report presents a statistical analysis of sources of error in the determination of ascorbic acid content. The quantitative contribution of each source of variation to the errors of the values required by the method is described and an estimate is made of the error

[1] This paper is the eleventh of a series from a cooperative investigation by the New York City Department of Health; the Division of Public Health Methods, National Institute of Health, United States Public Health Service; the Cornell University Medical College, Department of Public Health and Preventive Medicine and Department of Pediatrics; and the Milbank Memorial Fund.

The cooperating agencies have been assisted in carrying out this investigation by the Work Projects Administration for the City of New York, Official Project No. 65-1-97-21 W.P. 24, "Medical Evaluation of Nutritional Status."

of the determined ascorbic acid content when the procedure used is most favorable in that certain potential sources of error are controlled.

A similar analysis of errors in the micromethod is to be published shortly. There is also in progress a report on comparisons of determinations by the photelometric methods and the titrimetric methods.

Method of Photelometric Determinations

The estimation of ascorbic acid is based upon the color change resulting from reduction of 2.6 dichlorophenol-indophenol. By means of the photoelectric colorimeter, objective measurements are made of the intensity of a light beam passed through a solution of the dye (dye blank) and of the light intensity when a solution containing ascorbic acid has been added to the dye solution. The diminution of color in the dye from the addition of ascorbic acid permits an increased transmission of light, and the difference in galvanometer readings of light intensities for the original dye solution and the dye plus a solution containing ascorbic acid affords a measure of the change in the amount of light transmitted. The quantitative relationship between the change in light transmission and the amount of ascorbic acid added to the dye has been established and is expressed in the formula $C = K\ (\log. G_s - \log. G_b)$, where G_s is a value read from the galvanometer scale for the dye with the specimen solution added, G_b is the galvanometer reading of the prepared dye solution before the specimen solution is added, K is the constant for a given volume of the solutions which expresses the relationship between reduction of color in the dye and amount of ascorbic acid, and C is the determined amount of ascorbic acid in the specimen solution. The galvanometer scale is in units from 1 to 100, and in the macromethod, readings are from the upper half.

The value for K may be determined by transposing the equation to read $K = \frac{C}{\log. G_s - \log. G_b}$ and using weighed amounts of solutions

of crystalline ascorbic acid for the specimen solution, so that the value of C is known.

In the Nutrition Study an Evelyn colorimeter was used, and tables of values for 2 – log. G which come with the instrument were substituted for common logarithms. When this substitution is made the difference computation is reversed[2] and (log. G_s – log. G_b) becomes (2 – log. G_b) – (2 – log G_s). These values for (2 – log. G) are designated as L values, and when substituted in the equations, the formulae become $K = \frac{C}{L_b - L_s}$ and $C = K\ (L_b - L_s)$. Throughout this report, L with the subscript b is used for the value for 2 – logarithm of the galvanometer reading on the dye blank and L_s for the value of 2 – logarithm of the galvanometer reading after addition of a specimen solution to the dye acetate solution. The L table used gave values for one-quarter units of the galvanometer scale and readings were made to the nearest one-quarter unit, although only units are marked on the scale.

It is apparent that each observation required for determining the ascorbic acid content of a specimen of plasma is a potential source of error in the final estimate of the content. Consideration of the above formulae will show that a basic source of error is in the readings made of light intensity on the galvanometer scale. The reliability of the measurement of the transmitted light has been studied for the three types of solutions involved: (1) the prepared dye blank; (2) the dye blank plus a prepared solution of crystalline ascorbic acid; and (3) the dye blank plus the plasma specimen. Observations on the first two solutions provide the constant (K) used in the plasma determination; and those on the first and third, together with K, enter into the plasma determination.

It is obvious that any error in the ratio, K, has an important influence on the accuracy of the determination of ascorbic acid concentration in plasma. Mindlin and Butler suggested that an average

[2] This is shown as follows: (2 – log. G_b) – (2 – log. G_s) = 2 – log. G_b – 2 + log G_s = log. G_s – log. G_b.

K value for a particular colorimeter should be obtained from several determinations made on sample solutions of ascorbic acid of different concentrations, and that the K value so derived for a particular colorimeter could be taken as a constant value. In the course of this Study, K values were obtained daily for a period of nine months, and an analysis of the variation of K under different conditions forms a large part of this report. Although the preparation of solutions of ascorbic acid each day to determine K adds to the laboratory work, our data indicate that this is necessary to keep the error of K at a minimum.

Evidence on another possible source of inaccuracy to be presented relates to the ratio $\frac{C}{L_b - L_s}$ at different concentration levels. How constant is this value throughout the range of ascorbic acid concentration found in human plasma?

Data Used for Analysis

The data utilized in the analysis of errors in the macromethod are, in large part, the daily laboratory records from routine use of the procedure in determining plasma ascorbic acid for over 2,000 high school pupils examined during the eight months from October, 1939 to June, 1940. On the basis of a preliminary analysis, it was decided to determine K each day. The laboratory record provides a galvanometer reading in duplicate or triplicate, usually the latter, on the dye blank for each day and on each of two or three prepared ascorbic acid solutions, usually at concentrations of 1.0, 1.5, and 2.0 mg. per 100 cc. Each reading for a dye blank was from a separate colorimeter tube and each reading for a given concentration was from an independently prepared tube containing the dye blank plus an aliquot of the ascorbic acid solution. On each laboratory record there was recorded the number of the dye stock from which the dye solution was prepared, and the date on which the solution was prepared. The dye acetate solution usually was prepared in quantity to last for several days, but was seldom used more than five days.

Data for the preliminary analysis provided galvanometer readings on prepared solutions of crystalline ascorbic acid at seven concentrations, ranging from 0.1 mg. to 2.0 mg. per 100 cc. These data are referred to in the following discussion as the initial or test series, and have been used chiefly to compare K values derived from lower concentrations than those used in the routine series.

Error of Galvanometer Readings

Dye Blank Readings. From the large series of independent duplicate galvanometer readings on the same dye blank, data are available to show the variation in readings and to estimate the error of a single measurement of light transmission as read from the galvanometer scale. Two readings each day were paired[3] and the difference in L values was obtained. A frequency distribution of differences centering around zero is obtained, since differences were taken in a constant order and the sign of the difference retained. The standard deviation of these differences indicates the extent of the variation in L_b values if several observations are made on the same dye blank and eliminates all variation between different dye solutions. On the assumption that the true L value for each dye blank is approximated by the average of the L values for duplicates, the variance of differences (S.D.^{2}) divided by 2 (since two observations entered into each difference) gives an estimate of the variance and from this of the standard deviation for L_b based on a single galvanometer observation on a dye blank.[4] The standard deviation of an L_b from errors of measurement is given in Table 1 for all routine data combined and separately for observations made on

[3] Only two galvanometer readings were used for days on which three had been reported. With the records filed chronologically, for successive days, differences with signs were taken for: (1) first minus second reading for one day; (2) second minus third for the following day; and (3) first minus third reading for third day; and this order then was repeated.

[4] This standard deviation of an L_b from a series of duplicate readings on different dye blanks is the average standard deviation of L_b for each pair from the mean L_b for the pair and is equivalent to the standard deviation of a series of observations made on the same dye blank.

Dye Stock	Number of Pairs of Readings	Standard Deviation (L) for Difference in Duplicates	Estimated Error of L_b	
			Variance e_b $\left(\frac{\sigma \text{ diff.}^2}{2}\right)$	Standard Deviation σ_{e_b}
All Dye Stocks	135	.00425	.00000902	.00300
Stock I—With 17 Dye Acetate Solutions	55	.00407	.00000828	.00288
5 Different Stocks—16 Dye Acetate Sol.	80	.00437	.00000953	.00309

Table 1. Estimated error of L for a single galvanometer reading on the dye blank in the macrocolorimetric method.

solutions from one dye stock used for several months and for five different stocks each of which was used for a relatively short period.

The standard deviation of a single L_b value[5] based on 270 observations, or 135 pairs of duplicates, was 0.00300. The error of the L_b value is small in relation to the actual value which varied around 0.300, and, conversely, the reliability of the galvanometer measurement of light intensity was high. Some error in reading the galvanometer no doubt occurred, but in this procedure several factors also may affect the measurement, especially any slight imperfection in the tube or interfering particles in the solution or left on the tube after cleaning. The error found for a dye blank reading has been termed the accidental error of L_b, and is designated σ_{e_b}.

Galvanometer Readings on Dye Plus Ascorbic Acid. From galvanometer readings on two colorimeter tubes in which samples from the same dye blank were mixed with an aliquot of the same prepared solution of ascorbic acid of known concentration, a series of differences between paired L_s values was obtained as described above for differences in L_b values. These differences in L_s values

[5] The dye blanks throughout the Study gave G readings between 50 and 55 with the exception of a few readings from 47 to 49, and a few from 56 to 58. The L values, therefore, ranged from 0.328 to 0.231. *Change* in light transmission is the significant measurement, and the density of the dye blank is not precisely controlled. G readings from 50 to 55 are recommended by Mindlin and Butler.

include the type of error described for L_b plus any variability in the reaction of the same dye to the added ascorbic acid. Errors in measuring the ascorbic acid aliquot into the dye solution also would affect the differences. In Table 2 there is shown the standard deviation of the differences between duplicate readings on different concentrations of ascorbic acid with dye, and the estimated standard deviation of L_s from a single reading.

The reliability of a single observation on the reduced dye was very high. The standard deviation of L_s was in every case less than that for the dye blank alone for the same series of solutions, although at 1.0 mg. per cent the error for L_s from five different dye stocks was very nearly as great as the error of L_b. This does not mean that there was less variability in the G readings, for this variability was slightly higher for the reduced dye. The galvanometer value increases as the dye is reduced, or as greater amounts of ascor-

Table 2. Estimated error of L for a single galvanometer reading on the dye solution plus a solution of crystalline ascorbic acid in the macrocolorimetric method.

Dye Stock and Ascorbic Acid Concentration	Number of Pairs of G Readings	Standard Deviation (L) for Differences in Duplicates	Estimated Error of L_s	
			Variance c_s $\left(\frac{\sigma \text{ diff.}^2}{2}\right)$	Standard Deviation σ_{e_s}
All Dye Stocks				
1.00 Mg. Per Cent	126	.00373	.00000695	.00264
1.50 Mg. Per Cent	126	.00347	.00000602	.00245
2.00 Mg. Per Cent	125	.00330	.00000545	.00233
Stock I— 17 Dye Acetate Solutions				
1.00 Mg. Per Cent	52	.00302	.00000457	.00214
1.50 Mg. Per Cent	56	.00287	.00000413	.00203
2.00 Mg. Per Cent	53	.00349	.00000609	.00247
5 Stocks— 16 Dye Acetate Solutions				
1.00 Mg. Per Cent	74	.00416	.00000863	.00294
1.50 Mg. Per Cent	70	.00388	.00000753	.00274
2.00 Mg. Per Cent	72	.00313	.00000490	.00221

bic acid are added, and the L value not only becomes smaller but also a unit difference in the G value has a decreasing L value as G increases. The slightly greater variability in G readings was more than compensated for by the smaller L value per G unit. The error of measurement of L_s (σ_{e_s}) for all data at 1.0 mg. per 100 cc. was 0.00264, at 1.5 mg. it was 0.00245, and at 2.0 mg. it was 0.00233. The magnitude of these errors indicates that the reaction of the same dye blank to a given amount of ascorbic acid was very stable.

Variation in K Values

Accidental Error of K. Since the K value[6] is a quotient derived from a known concentration of ascorbic acid and the difference ($L_b - L_s$), the error of a single K from accidental errors in determining L_b and L_s may be determined from the above estimates of the accidental errors of L_b and of L_s. The accidental error of this difference (L_d) is the square root of the sum of the variances of the two values, from the formula σ diff. $= \sqrt{\sigma_1^2 + \sigma_2^2}$. The approximate error of K from this error for L_d may be determined from the formula for the standard deviation of a quotient (3). In this instance, the dividend C, the known concentration, is not a variable but a fixed value for which the error is only that incidental to preparing the solution. If the error for C is assumed to be zero, the standard deviation of a calculated K is obtained as follows:[7]

$$\sigma_K = \frac{C}{L_d}\left(\frac{\sigma_{e_d}}{L_d}\right)$$

[6] The K value, as given in this report, is the constant obtained when C for the 4 cc. of a standard solution used in the determination is taken as the equivalent concentration per 100 cc. of plasma filtrate.

[7] The formula (3) for the standard deviation of a quotient is:

$$\sigma_{X_1/X_2} = \frac{X_1}{X_2}\sqrt{C.V._1^2 + C.V._2^2 - 2r_{1,2}\,C.V._1\,C.V._2}$$

It is reasonable to assume no correlation because the standard deviation to be estimated is for a K value for a specific ascorbic acid solution, and is that associated with errors of

(Continued on page 186)

Ascorbic Acid Solution Mg. Per 100 cc.	Accidental Error of L_d $\sigma_{e_d} = \sqrt{(\sigma_{e_b})^2 + (\sigma_{e_s})^2}$	True L Difference L_d When		Coefficient of Variation C.V. $= \sigma_{e_d} \div L_d$		Error of K from σ_{e_d} $\sigma_K = K \times C.V.$	
		K = 9.7	K = 9.2	K = 9.7	K = 9.2	K = 9.7	K = 9.2
1.00 Mg.	.00400	.103	.109	.0388	.0367	.376	.338
1.50 Mg.	.00388	.155	.163	.0250	.0238	.243	.219
2.00 Mg.	.00380	.206	.217	.0184	.0175	.178	.161

Table 3. Error of estimate for K attributable to accidental error of a single observation for the dye blank and of a single observation for the ascorbic acid solution at three different concentrations. Estimate is derived from data in Tables 1 and 2.

It is apparent from this formula that the size of the standard deviation of K will vary positively with the magnitude of K since it is the product of K and the coefficient of variation of the L difference, or $\sigma_{e_d} \div L_d$. An example of the calculation of the standard deviation of a K value, when only the effect of the accidental error of L_b and of L_s is measured, follows for K derived from a single observation for L_b and for L_s.

(Table 1) variance for L_b $= .00000902$
(Table 2) variance for $L_{1.0}$ mg. $= .00000695$
Variance of L_d Sum $= .00001597$

$$\sigma_{e_d} = \sqrt{.00001597} = .00400$$

If L_d for 1.0 mg. $= .100$, then $K = \frac{1.00}{.100} = 10.0$

Standard deviation of K $= 10.0 \left(\frac{.00400}{.100}\right)$, or $10.0 \times .0400 = .400$

In Table 3, the estimated error of K attributable to the accidental variability of L_b and L_s is shown for three concentrations of ascorbic

measurement of L_d (σ_{e_d}) for a constant solution. For the standard deviation of K, the formula is:

$$\sigma_K = \frac{C}{L_d}\sqrt{\left(\frac{\sigma_e}{C}\right)^2 + \left(\frac{\sigma_{e_d}}{L_d}\right)^2}$$

but if σ_e is taken as 0, $\sigma_K = \frac{C}{L_d}\sqrt{\left(\frac{0}{C}\right)^2 + \left(\frac{\sigma_{e_d}}{L_d}\right)^2}$ or $\frac{C}{L_d}\left(\frac{\sigma_{e_d}}{L_d}\right)$

acid when K is at two different levels. It is clear that this error in K varies inversely with the concentration from which it is derived. Thus, if K is 9.7, the standard deviation of K is 0.376 at 1.0 mg. per 100 cc., but it is 0.178 at 2.0 mg. Most of this decrease in the error as the concentration increases results from the fact that the difference, L_d, must increase proportionately with the concentration if K is the same at each concentration and the coefficient of variation becomes smaller unless the standard deviation of L_d also increases proportionately. But in Table 3, it is shown that the standard deviation of L_d decreased slightly, as the concentration was increased. From these data, it is apparent that the error of K from these accidental sources is a minimum if K is derived from high concentrations of ascorbic acid within the range of ascorbic acid values to be expected in the material to be analyzed.

The accidental error of K estimated in Table 3 is that which is shown in variability of observations on a dye blank at a given time and of observations on this blank reduced by aliquots of the same prepared ascorbic acid solutions. It is, therefore, the apparent error of K within one day. The true error of K for any day will include the error in C which results from preparing the ascorbic acid solutions. On each day 100 mgs. of ascorbic acid were weighed for the preparation of a stock solution. From this stock solution, independent dilutions were made for the three standard solutions with concentrations equivalent to 2.00, 1.50, and 1.00 mg. per 100 cc. No experimental data were collected to estimate the error in preparing these solutions. However, K values obtained from independently prepared solutions will vary as a result of errors in the solution as well as from the accidental error described. This total accidental variation in K from errors of C and of L_d is found in K values derived on different days. The accidental error of L_d is known, and the error in the solutions can be estimated from the observed day-to-day variability of K if the effect of other factors on the variation in K can be controlled.

The same dye acetate solutions were used for several days, and it has been assumed that, if the standard deviation of L_b for different days on which a given dye solution was used did not exceed that found for the accidental error of L_b, the K values for the different days also should vary within limits of the accidental error of L_d plus the error of the prepared solution. For many dye acetate solutions, the observed standard deviation of L_b for the entire period of use was significantly higher than the accidental error of L_b, and this will be discussed later. There were nine dye solutions used from three to six days for which the standard deviation of L_b was from .00224 to .00332, as compared with the estimated accidental error of a single L_b of .00300. For each of these nine dye acetate solutions, the excess variation in K over that attributable to accidental errors of L_b and L_s is assumed to be due to errors in solutions prepared each day.

The method of estimating the error of C from the observed variation of K for each dye acetate solution and the accidental error of L_d for each dye solution was to substitute these known values in the formula for the standard deviation of K and solve for the unknown value, the error of C. The formula to determine the error of C derived from the formula for the standard deviation of the quotient K, is obtained as follows:

$$\sigma_K = K\sqrt{\left(\frac{\sigma_c}{C}\right)^2 + \left(\frac{\sigma_{e_d}}{L_d}\right)^2}$$

$$\therefore \quad \frac{\sigma_K}{K} = \sqrt{\left(\frac{\sigma_c}{C}\right)^2 + \left(\frac{\sigma_{e_d}}{L_d}\right)^2} \text{ and } \left(\frac{\sigma_c}{C}\right)^2 = \left(\frac{\sigma_K}{K}\right)^2 - \left(\frac{\sigma_{e_d}}{L_d}\right)^2$$

where σ_K is the observed standard deviation of K for different days on which the same dye solution was used, σ_{e_d} is the error of the difference (L_d) from the accidental error of L_b for this dye solution and the accidental error of L_s obtained for this dye solu-

tion. The square of the coefficient of variation $\left(\frac{\sigma_c}{C}\right)^2$ was determined for each dye solution by the following calculations:

1. The mean variance of L_s from accidental errors for each specific dye acetate solution was calculated as the *within-the-day* variance from triplicate readings each day and was determined for each of the concentrations.
2. The variance of L_b for all observations on a specific dye solution was used as the variance of a dye blank reading from accidental errors.
3. The variance of the L difference between mean L_b and mean L_s for one day from accidental errors (σ_{e_d}) was calculated for each dye solution as the sum of variances of L_b and L_s divided by 3 since three independent readings were taken each day on the dye blank and on the dye plus ascorbic acid solution.
4. The variance of L_d for each day at a specific concentration was divided by the square of mean L_d at that concentration for all days on which the dye solution was used. This value is $\left(\frac{\sigma_{e_d}}{L_d}\right)^2$ in the above formula.
5. For K values on different days for the same dye solution, the mean K and the standard deviation of K was obtained at each concentration of ascorbic acid. From these, the value $\left(\frac{\sigma_K}{K}\right)^2$ for the above formula was obtained.
6. The difference, $\left(\frac{\sigma_K}{K}\right)^2 - \left(\frac{\sigma_{e_d}}{L_d}\right)^2$ obtained for each dye solution was weighted by the number of days of use and the mean difference taken as the estimate of the square of the coefficient of variation of C.

The mean percentage errors of C obtained from the nine dye acetate solutions used for varying periods over a total of thirty-eight different days were:

For C = 1.00 mg., the percentage error = 2.94[8]
For C = 1.50 mg., the percentage error = 1.90
For C = 2.00 mg., the percentage error = 1.46

For the nine solutions, the variation in the estimated percentages

[8] This estimated percentage error in solutions of 1.0 mg. per cent is very close to an

(Continued on page 190)

was small. These estimates of the error in C are believed to be reasonably good approximations of the percentage variation in C to be expected over a period of time. On any one day, the chances are nearly two to one that the error in C is less than the estimated mean percentage.

The effect of the percentage error of C on the total accidental error of K for one day is not reduced by repeated observations on each prepared solution, since aliquots of the same solution are used in making these observations on the dye blank plus ascorbic acid solution. The accidental error of L_b and L_s is reduced by taking several observations. An estimate of the expected error of K from accidental sources discussed may be expressed as follows:

$$\sigma_{e_K} = \frac{C}{L_d}\sqrt{\left(\frac{\sigma_e}{C}\right)^2 + \left(\frac{\frac{V_{e_b}}{n_b} + \frac{V_{e_s}}{n_s}}{L_d^2}\right)}$$

In this formula, σ_{e_K} is the expected total accidental error of K, V_{e_b} is the square of the estimated error of measurement for L_b, n_b is the number of independent galvanometer readings taken on the dye blank, V_{e_s} is the square of the error of measurement for L_s, n_s is the number of readings on the standard ascorbic acid solution, and L_d is the difference between the mean L_b and mean L_s.

In the present Study, as previously stated, it was usual to make three readings on the dye blank each day and three readings with the standard solution added to obtain a K value at 1.0 mg., 1.5 mg., and 2.0 mg. concentrations. The estimates of variances of L_b and L_s from accidental errors, shown in Tables 1 and 2, and the percentage

estimate made from data from a series of observations on plasma to which a known amount of ascorbic acid was added. To ninety-seven plasma specimens, an amount of ascorbic acid equivalent to 1.0 mg. per cent was added, and the plasma content was determined before and after this addition. The standard deviation of the differences between the initial determinations and those after addition of ascorbic acid was found to be greater than that for duplicate determinations when no addition was made. If the difference between the variance with addition of 1.0 mg. per cent ascorbic acid and the variance without addition is assumed to be the variance contributed by errors incidental to weighing and handling the crystalline ascorbic acid, then the estimated standard deviation for the 1.0 mg. per cent solution added to each specimen was 2.5 per cent.

errors for C may be substituted in the above formula to derive the expected total error of K at each concentration.

The expected total accidental errors of daily K values for each of the three concentrations and at two levels for K have been computed and are shown in Table 4. These estimates of the error of the daily K should approximate the observed standard deviation of K over a period of time unless other factors caused the K to change. However, the average value of K on dye stock I was about 9.2, but the observed standard deviation on 1.0 mg. per cent solutions was 0.379 as compared with the expected standard deviation from accidental errors of 0.334, and on 2.0 mg. per cent, the observed standard deviation was 0.211 as compared with the expected standard deviation of 0.163. Further analysis of the variation in K was made, therefore, to identify, if possible, other factors which affected the K value.

K Variation for Different Dye Solutions. Inspection of the routine laboratory records suggested that K changed when new dye solutions were used. Therefore, an analysis of the variation in daily K values was made to determine the magnitude of this variation under three conditions; namely, when the same dye acetate solution

Table 4. Total error of measurement for K estimated from percentage error for prepared ascorbic acid solutions plus accidental error of L difference when three observations are made for the dye blank and for the blank plus solution.[1]

Ascorbic Acid Solution	$\frac{\sigma_e}{C}$	$\frac{\sigma_{e_d} + L_d^*}{\sqrt{3}}$		Total Accidental Error of K or σ_{e_K}	
		K = 9.7	K = 9.2	K = 9.7	K = 9.2
Mg. Per 100 cc.	(1)	(2)	(3)	$\sqrt{(1)^2+(2)^2}\times K$	$\sqrt{(1)^2+(3)^2}\times K$
1.00 Mg.	.0294	.0224	.0212	.359	.334
1.50 Mg.	.0190	.0145	.0137	.232	.216
2.00 Mg.	.0146	.0107	.0101	.176	.163

[1] See text, pages 189-190.

*Since n = 3 for both L_b and L_s, the coefficient of variation (C.V.) for L_d in Table 3 when n is 1 may be divided by $\sqrt{3}$ to obtain C.V. for L_d from three observations.

was used for several days; when different dye solutions prepared from the same dye stock were used; and when different dye stocks were used. The basic data for the analysis included all routine daily laboratory records for the five dye lots from which at least three different dye acetate solutions were prepared. The analysis is made separately at each of the three levels of ascorbic acid solutions in order to eliminate different concentration levels as a source of variation in K. The mean daily K values at each concentration based on three galvanometer readings on the dye blank and three readings after addition of ascorbic acid were used for a variance analysis to show the mean variation of these daily K values for days on which the same dye acetate solution was used, and to compare this variance with the variance for means of the K values derived from each dye acetate solution prepared from the same dye lot. The results of this analysis of K values derived from concentrations of 1.0 mg., 1.5 mg., and 2.0 mg. per 100 cc. are shown in Table 5.

The total variance of all K values obtained on the same dye stock was in most instances greater than the mean variance of K on days for which the same dye acetate solution had been used. This greater total variance reflects the fact that, as shown in Table 5, the variance among mean K values for different acetate solutions was frequently, although not consistently, greater than the variance of K within dye solutions. In ten of the fifteen comparisons, the variance among mean K values for different dye solutions of a single stock is greater than that for daily K values for the same dye solutions; and in the five instances in which the reverse occurred, the variances did not differ significantly. In seven of the ten cases of greater variance among means for specific dye acetate solutions, the statistical probability is less than 5 chances in 100 that this greater variance would occur as a result of the variance of K on the same dye solution. Six of the eight comparisons for which the variance among solutions was not significantly greater than within solutions are for the two dye lots from which only three acetate solutions were prepared (2

Table 5. Significance of variations in K associated with different dye acetate solutions prepared from various dye stocks, by dye stock and concentration of ascorbic acid. Each K is based on three L_b values and three L_a values.

Source of Variation (Dye Acetate Solution = D.A.)	Mean K	Variation of K			Variance Ratio F	P
		D.F.	Mean Square	S.D.		
Dye Lot I	**1.0 MG. PER CENT CONCENTRATION**					
Total	9.16	45	0.1435	.379		
Among D.A. Means		12	0.2057		1.70	>.05
Within D.A. Sol.—Days		33	0.1209	.348		
Dye Lot III						
Total	9.29	25	0.1693	.411		
Among D.A. Means		3	0.1669		1.02*	>.05
Within D.A. Sol.—Days		22	0.1696	.412		
Dye Lot V						
Total	9.35	12	0.1252	.354		
Among D.A. Means		2	0.1915		1.71	>.05
Within D.A. Sol.—Days		10	0.1120	.333		
Dye Lot VI						
Total	9.45	25	0.2873	.536		
Among D.A. Means		5	0.6411		3.47	.01–.05
Within D.A. Sol.—Days		20	0.1845	.430		
Dye Lot VII						
Total	9.70	11	0.4454	.667		
Among D.A. Means		2	0.2535		1.93*	>.05
Within D.A. Sol.—Days		9	0.4881	.699		
Dye Lot I	**1.5 MG. PER CENT CONCENTRATION**					
Total	9.24	46	0.0822	.287		
Among D.A. Means		12	0.1435		2.37	.01–.05
Within D.A. Sol.—Days		34	0.0606	.246		
Dye Lot III						
Total	9.40	24	0.0986	.314		
Among D.A. Means		3	0.2417		3.09	.01–.05
Within D.A. Sol.—Days		21	0.0782	.280		
Dye Lot V						
Total	9.37	13	0.0530	.230		
Among D.A. Means		2	0.0436		1.25*	>.05
Within D.A. Sol.—Days		11	0.0547	.234		
Dye Lot VI						
Total	9.58	25	0.1773	.421		
Among D.A. Means		5	0.5651		7.90	<.01
Within D.A. Sol.—Days		20	0.0715	.267		
Dye Lot VII						
Total	9.70	11	0.2006	.448		
Among D.A. Means		2	0.0824		2.75*	>.05
Within D.A. Sol.—Days		9	0.2268	.476		
Dye Lot I	**2.0 MG. PER CENT CONCENTRATION**					
Total	9.34	41	0.0444	.211		
Among D.A. Means		11	0.0884		3.13	<.01
Within D.A. Sol.—Days		30	0.0282	.168		
Dye Lot III						
Total	9.39	25	0.0507	.225		
Among D.A. Means		3	0.1468		3.91	.01–.05
Within D.A. Sol.—Days		22	0.0376	.194		
Dye Lot V						
Total	9.49	9	0.0260	.161		
Among D.A. Means		2	0.0350		1.49	>.05
Within D.A. Sol.—Days		7	0.0235	.153		
Dye Lot VI						
Total	9.59	24	0.1582	.398		
Among D.A. Means		5	0.4281		5.17	<.01
Within D.A. Sol.—Days		19	0.0828	.288		
Dye Lot VII						
Total	9.77	11	0.0927	.304		
Among D.A. Means		2	0.0750		1.29*	>.05
Within D.A. Sol.—Days		9	0.0967	.311		

* The greater mean square is that *within* dye acetate solutions.

degrees of freedom). When K was derived from solutions of 1.5 and 2.0 mg. per cent, the variance among dye solutions was significantly greater than that within solutions for each of the three dye lots from which four to twelve solutions had been prepared. In general, it can be said that there is a definite tendency for K values for different dye acetate solutions of the same dye lot to differ significantly.

It seemed, *a priori*, that the variance of K for days when the same dye solution was used should be approximately that which would result from the accidental errors of measurement of L_b and L_s, and of the concentration (C). Thus, the standard deviations shown in Table 5 for K *within* dye solutions should be similar to the estimates given in Table 4. However, in nearly every instance, the observed standard deviation within dye solutions was higher than the estimated expected standard deviation from accidental errors. The magnitude and consistency of a variation for K within dye acetate solutions in excess of that from measured accidental errors suggested that some other factor affected the K on different days.

The only new factor introduced in the determination of K on different days for which the same dye acetate solution was used was that of time. As previously mentioned, the dye blank values from day to day on the same dye acetate solution varied more than would be expected from the accidental error of L_b. The accidental error of L_b was found to be .0030, and the standard deviations for L_b within dye solutions for several dye stocks were as follows: Stock I, .0050; Stock III, .0063; Stock V, .0040; Stock VI, .0041; and Stock VII, .0047. In order to test whether K changed if the L_b changed for the same dye solution after several days of use, the K values obtained on the first and last day for the same dye acetate solution were compared and the difference in K was then related to the difference in L_b on the first and last day. The *differences* between K on the first and last day (last day – first day) for the same dye acetate solution are plotted in Figure 1, for twenty-five

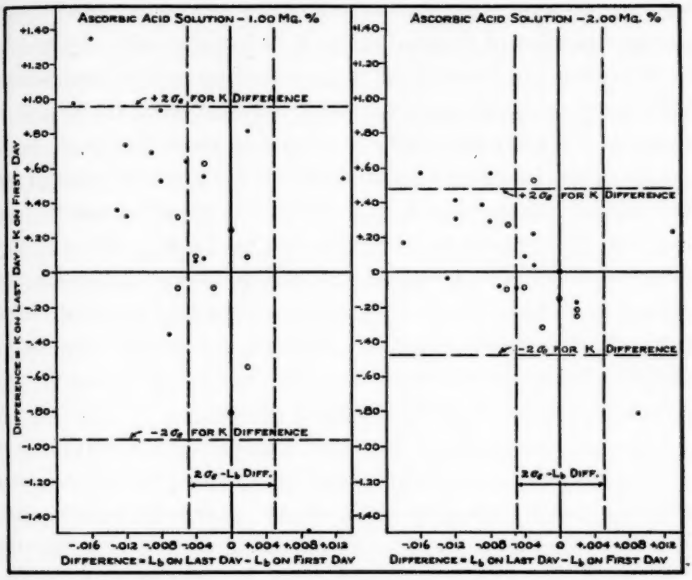
Ascorbic Acid Solution – 1.00 Mg. %
Ascorbic Acid Solution – 2.00 Mg. %
+2σd for K Difference
−2σd for K Difference
2σd – Lb Diff.
Difference = K on Last Day – K on First Day
Difference = Lb on Last Day – Lb on First Day

Fig. 1. Change in K values derived from same dye acetate solution after three or more days of use and the associated change in L value for dye solution. Approximate limits for twice the standard deviation expected on the basis of accidental errors of measurement are shown for a difference between two K values and for a difference between two L values for a dye blank.

dye acetate solutions and the associated difference between L_b on the first and last day also is shown. One dye solution was used for sixteen days; seventeen solutions were used from five to eight days, and the other seven were used only three or four days.

It is evident in Figure 1, that there was a definite tendency for K to increase after the dye solution stood for several days, and this increase obviously was correlated with a decrease in the L_b value.[9] If the differences in K were the result of random association of the accidental errors and the reaction of the dye blank to ascorbic acid

[9] Open circles in Figure 1, indicate K differences and L_b differences for the nine dye acetate solutions used to determine the percentage error of C. The differences in K shown for these solutions are not skewed toward high positive values and therefore do not seem to be affected by a change in the dye reaction to ascorbic acid.

had been constant regardless of its L value, we should expect about an equal number of plus and minus K differences, with all but one or two values within the limits of the dotted horizontal lines. Minus differences, with one exception, were less than twice the expected standard deviation of a difference,[10] that is, above the lower horizontal dotted line; but plus differences for K, both at 1.0 and 2.0 mg. concentration, were skewed toward the upper horizontal line and two differences were above the line for 1.0 mg. solutions and three were above the line for 2.0 mg. solutions. Although the change in K with time was not consistent for all dye acetate solutions, the data strongly suggest a tendency for the dye solution to diminish in density and to show a small change in the reaction to ascorbic acid when kept for a number of days.

It is outside the scope of this report to attempt any interpretation of this change in dye reaction to ascorbic acid after the dye solution stood for several days. Time alone may not have been the real cause. However, the change was a significant factor in increasing the variation in K values for some dye solutions. In order to eliminate this factor as a source of error in K, a K value derived for the specific day on which determinations are to be made seems essential.

Measurement of the reduction in density of a specific dye by a given amount of ascorbic acid is the critical value for determining a K which will furnish an accurate estimate of ascorbic acid in a plasma specimen. Therefore, the accuracy of the plasma determination depends on eliminating as far as possible the effect of sources of change in the K value. Different dye solutions have been found to react quite differently to ascorbic acid and even the same solution sometimes was not constant over a period of several days. From the

[10] The approximate expected standard deviation of a difference between two daily K values is estimated from the total accidental error of a daily K value shown in Table 4, and is $\sqrt{2}$ times σ_{e_K}.

The σ_{e_K} used was intermediate between those given for K equals 9.2 and 9.7. A more precise test of the significance of each difference based on data specific for each dye solution probably would show a greater number of differences to be larger than twice their standard deviation.

foregoing analysis, it is possible to generalize that, unless K is obtained for each day, large errors may be introduced into the plasma determinations.

Since dye solutions from the same dye stock frequently gave different K values, it is not surprising that different dye stocks were characterized by quite different K values. The mean K values at three concentrations for five different dye stocks are shown in Table 5. The range for mean K values on 1.00 mg. per cent solutions was 9.16 to 9.70; on 1.50 mg. per cent solutions was 9.24 to 9.70; and on 2.00 mg. per cent solutions 9.34 to 9.77. It is significant to note that changes in the mean K values with use of a different dye stock were approximately the same for K at each concentration. The variation among means for these different dye stocks is beyond the statistical limits of likelihood for random samples drawn from a homogeneous population of dye stocks. For some reason, these dye stocks did not react similarly to the addition of ascorbic acid, or, in other words, the reduction in dye density was not constant for a given amount of ascorbic acid. Furthermore, the variation in K values differed for these dye stocks and the reaction of some stocks apparently was much more variable than that of others.

Differences in K Derived from Different Concentrations. The mean K values for each dye stock derived from ascorbic acid solutions of three different concentrations, shown in Table 5, indicate a definite tendency for K to increase slightly as the concentration was increased. If K changes significantly at different concentrations, it obviously is important to know the magnitude of the difference in K for the range of concentrations to be expected for plasma or other materials being assayed. Present use of the method indicates acceptance of K as a constant value for all levels of ascorbic acid or as differing so slightly as to have no important effect on the accuracy of the determination.

The mean K values for the entire routine series are given in

Concentration of Ascorbic Acid in Prepared Solution	Routine Laboratory Series		Initial Series	
	Number of Days	Mean K	Number of Days	Mean K
0.10 Mg. Per Cent			20	10.02
0.30 Mg. Per Cent			20	9.38
0.50 Mg. Per Cent			20	9.35
0.75 Mg. Per Cent			20	9.60
1.00 Mg. Per Cent	127	9.31	20	9.56
1.50 Mg. Per Cent	127	9.40	20	9.62
2.00 Mg. Per Cent	127	9.45	20	9.67

Table 6. Comparison of mean K values derived from prepared ascorbic acid solutions of different concentrations.

Table 6 for K at 2.0 mg., K at 1.5 mg., and K at 1.0 mg. per cent. From these means, differences between any two levels are readily obtained. The method used to test whether the difference between the mean K values derived from two solutions of different concentrations is statistically significant was to obtain the standard error of this difference from the differences between K values for two concentrations on the same day. The two values compared are based on the same dye blank and the concentrations are from the same solution of weighed crystalline ascorbic acid, although a slight error for separate additional dilution is involved, and the differences, therefore, are not affected by the various sources of large variability in K which have been discussed. Each of the mean differences between K values at two concentration levels is significant statistically for the routine series. For concentration 2.0 mg. per cent, the K value was, on the average, 0.14 higher than for concentration 1.0 mg. per cent. In view of the large amount of data on which this finding is based, it can be concluded that K is not constant at all levels of ascorbic acid and that the use of K derived from one concentration, or the average of several concentrations, introduces a constant error in specimen determinations with concentrations differing from that on which K was derived. It is important, therefore, to determine the magnitude of the differences between the true K values for various concentrations, especially between

the low concentrations of interest in plasma determinations and higher concentrations customarily used for deriving K.

From the initial experimental data, previously mentioned, a relatively small amount of data are available for concentrations of 0.1 mg., 0.3 mg., 0.5 mg., and 0.75 mg. per cent, in addition to 1.0, 1.5, and 2.0 mg. per cent. Two determinations on each level were made on twenty days, and from these data, the mean K values at the various levels are given in Table 6. The K values for the seven concentrations show a definite tendency to increase as the ascorbic acid concentration is increased, with the exception that on 0.1 mg. per 100 cc. the K value is highest and therefore entirely out of line. At 0.75, 1.00, and 1.50 mg. per cent in this initial series, the K values do not differ significantly; but they are very significantly higher than those at 0.3 and 0.5 mg. per 100 cc. and at 1.5 mg. per cent, K is significantly lower than at 2.0 mg. per 100 cc. These data show the same trend in differences as that found for the routine daily records, but the difference between K at 1.0 and at 1.50 mg. is somewhat greater for the routine series than in the initial series. At 0.1 mg., the variability of K from accidental error of L_a is very large and the likelihood of a high percentage error in the solution is great; therefore, a larger number of carefully made observations is needed to obtain an accurate K at this level. For the remainder of the range of ascorbic acid solutions, these data strongly support a conclusion that a constant K relationship between the measured reduction of dye density and the amount of ascorbic acid does not hold precisely for all levels of ascorbic acid concentration.

The practical importance of the constant bias in the determination of ascorbic acid in a specimen with ascorbic acid content different from that of the solutions from which K was derived depends on the magnitude of the error introduced and the accuracy desired. The maximum difference in mean K values was 0.32 for 0.5 mg. and 2.0 mg. solutions. This is about 3 per cent of the K value and a K derived from 2.0 mg. solutions would overestimate

ascorbic acid content at 0.5 mg. by this per cent, or .015 mg. An error of this size, on the average, is not important in routine determinations. For some experimental work, any overestimate or underestimate in a determination may have significance as, for example, in comparison of determinations by two different methods.

The differences in K derived from solutions with different concentrations have a bearing on the choice of which concentrations furnish the best K for plasma determinations. The bias of overestimation of low plasma values if K from high concentrations is used is small in absolute amounts of ascorbic acid and this error is more than offset by the much smaller accidental error of K at high levels. Furthermore, K from high concentrations results in a conservative estimate of low plasma values.

Summary Discussion of K as a Source of Error in Plasma Determination

The foregoing analysis of sources of variation in K values has provided evidence that, for the greatest accuracy, K should be determined each day. Data presented have shown that the mean K values derived from five different dye stocks varied from 9.16 to 9.77; and the characteristic K for any stock must be determined. Furthermore, for different dye acetate solutions from the same dye stock, the mean K values frequently varied more than would be expected if they were means of homogeneous samples affected only by the factors which cause variability in K when the same dye acetate solution is used for several days. Finally, even when the same dye acetate solution was used, the K values obtained for different days showed a tendency for K to be significantly higher after the dye solution had been used for several days than it was on the day on which a fresh dye solution was prepared. These sources of variation in K, namely, different dye stocks, different dye acetate solutions, and the effect of time on a dye solution, can be controlled if K is determined each day from prepared solutions of ascorbic acid.

For a K value determined for a dye solution at a given time and from a single ascorbic acid solution, four sources of error have been discussed, as follows:

1. Dye blank reading, L_b. The standard deviation of a single observation on the dye blank was found to be, in L values, 0.00300.

2. Dye density reading, L_s, after addition of ascorbic acid solution to dye blank. The error in L_s readings varied somewhat for different concentrations; at 1.00 mg. per cent, the standard deviation of L_s for a single observation was 0.00264; at 1.50 mg. per cent, it was 0.00245; and at 2.00 mg. per cent, it was 0.00233.

3. Percentage error in ascorbic acid solutions. The estimated error in the concentration of prepared solutions, C, is 2.94 per cent for 1.00 mg. solutions, 1.90 per cent for 1.50 mg. solutions, and 1.46 per cent for 2.00 mg. solutions.

4. Differences between K values derived from solutions with different concentrations. K values were highest if derived from 2.00 mg. per cent solutions and consistently decreased as the concentration was reduced. K at 2.00 mg. was, on the average, 0.14 higher than at 1.00 mg. Insufficient data are available for reliable estimates at lower concentrations, but K continued to decrease.

The first three sources of error contribute to a total accidental error of K on any one day for a specific concentration. The fourth source of error is effective only as K for a specific concentration is applied in determinations for specimens with a different concentration.

The standard deviation of K from all accidental sources for one day decreases as the concentration from which it is derived increases and it increases slightly as the K value increases. With three readings on the dye blank and three readings on the measurement of dye reduction by the ascorbic acid solution, the error of measurement of K with a value 9.2 is estimated to be approximately 0.334 on solutions of 1.00 mg. per cent, 0.216 on solutions of 1.50 mg., and 0.163 on solutions of 2.00 mg. per cent. About two-thirds of the variance, or square of this standard error of K, is contributed by the error in preparing the solutions of ascorbic acid.

The use of an average K derived each day from several solutions with different concentrations does *not* reduce the error of K for several reasons. In the first place, if each solution is a dilution of the same solution of a single weighed amount of crystalline ascorbic acid, as it was in this Study, error in the master solution is carried over into the dilutions and is not averaged. By the use of two or more independently prepared master solutions for one day, the error of K could be reduced; but this does not seem necessary since the error is not large. In the second place, the accidental error of K increases as the concentration is reduced, and the average error of K from several concentrations will be intermediate between that for the highest concentration and that for the lowest concentration. Finally, since K is not constant at each concentration, the mean K from several concentrations is accurate only for some specific intermediate concentration, approximately the average of the concentrations used. Furthermore, the observed variability of the K values from which the mean is derived does not afford a measure of the true error of the mean K.

The true error of a mean K based on several ascorbic acid solutions of different concentrations is the mean of the variances of the measurement errors at each concentration. This eliminates the effect of differences between K values for different concentrations. The standard error of mean K when K is determined at concentrations of 1.00 mg., 1.50 mg., and 2.00 mg. is shown below, and this error is compared with that for mean K derived only on the two higher concentrations. Since K differs slightly at each concentration, L_d also is not exactly proportional to the difference in concentrations used. With adjustment for the mean trend in K with the concentration and n equals 3 for L_b and for L_a, the standard error of mean K becomes:[11]

If C = 1.00 and K = 9.31; $\sigma_{e_K} = 9.31 \times .0364 = 0.339$

[11] The calculation is from the formula on p. 190 for σ_{e_K}. The L_d values at each concentration are given in Table 7.

If C = 1.50 and K = 9.40; $\sigma_{e_K} = 9.40 \times .0236 = 0.222$
If C = 2.00 and K = 9.45; $\sigma_{e_K} = 9.45 \times .0179 = 0.169$
Mean K centering at about 1.50, $\sigma_{e_K} = 9.39 \times .0271 = 0.254$
Mean K centering at about 1.75, $\sigma_{e_K} = 9.425 \times .0210 = 0.198$

Thus, the standard error of mean K for one day from accidental sources is 0.254 if solutions of 1.00, 1.50, and 2.00 mg. per cent are used, and it is 0.198 if the two higher solutions are used. The percentage error in K derived from solutions with 2.00 mg. per 100 cc. was only half as great as that for solutions of 1.00 mg., and the standard error of a mean K based only on solutions of 1.50 and 2.00 mg. per cent is appreciably lower than that for a mean K including the 1.00 mg. solution.

Error in Determination of Ascorbic Acid in Plasma

The concentration of ascorbic acid in the plasma filtrate is equal to K ($L_b - L_s$), where L_b is the L value for the dye blank and L_s is the L value for the galvanometer reading of the dye solution with filtrate added. The error of the determined concentration in the filtrate is that attributable to the error of K plus that due to the error of measurement of reduction in dye density ($L_b - L_s$) by the plasma filtrate. The standard deviation of a determined concentration of ascorbic acid in plasma which is expected from total errors of measurement[13] is given in Table 7. The only new value needed for estimating the error of a plasma concentration (C_2) is the accidental error of L_s for filtrates. Data are available for duplicate readings on plasma filtrates from which an estimate of the standard deviation of a single observation was made. The standard deviations of L_s at four levels for plasma are shown in Table 7. The error of measurement of L_s when plasma filtrate is added to the dye is somewhat higher than the error of measurement of L_s when prepared solutions are used.

[13] The standard deviation for the ascorbic acid concentration in plasma, C_2, derives from

(Continued on page 204)

Concentration True C to be Determined Mg. Per 100 cc.	K Value For Specific C	L Difference Expected for C	Error of Single Galvanometer Reading on Dye + Filtrate σ_{e_2}	C Mean Value Obtained for K = 9.425	Percentage Error of C	Standard Deviation of C Value, About Mean C
0.40	9.15[1]	.0437	.0038[2]	.412	.097	.040
1.00	9.31	.1074	.0029[3]	1.012	.035	.035
1.50	9.40	.1596	.0027[4]	1.504	.026	.040
2.00	9.45	.2116	.0026[4]	1.994	.022	.044

[1] Estimated from difference in K values in initial series between mean at 0.30 and 0.50 mg. per cent and 1.50 mg. per cent; other K values from routine data.
[2] From fifty-one pairs of duplicate observations on plasma with C of .20–.59 mg. per cent.
[3] From thirty-seven pairs of duplicate observations on plasma with C of .80–1.19 mg. per cent.
[4] Estimated as 20 per cent higher variance than for prepared ascorbic acid solutions (Table 2) on basis of ratio at 1.00 mg. per cent.

Table 7. Reliability of concentration values of ascorbic acid in plasma determined on the Evelyn colorimeter by the macromethod with K derived from solutions of 1.50 mg. and 2.00 mg. per cent and based on three galvanometer readings for the dye blank and for each solution and one reading on the dye filtrate.

The error estimated for a determination of ascorbic acid in plasma is given in Table 7 for concentrations of 0.40 mg. per cent, 1.00 mg., 1.50 mg., and 2.00 mg. The error is that to be expected when K is derived from standard solutions of 1.50 mg. and 2.00 mg. per cent, or an average concentration of 1.75 mg. per cent. If lower concentrations are used for K, the error will be larger. The standard deviations for the determined ascorbic acid content indicate the expected variability of determinations for plasmas with equal ascorbic acid content, but this is the variability around a mean

both a product and a quotient value, as is seen if K $(L_b - L_e)$ is expanded for the K factor to read:

$$C_2 = \frac{C_1}{L_{b_1} - L_{e_1}} (L_{b_1} - L_{e_2})$$

where C_1 is the concentration of the standard solution used. The standard deviation for the product-quotient value (C_2) was obtained from the formulae for a product and a quotient (3) and was adjusted for an approximate correlation value that results from a constant L_b value for the standard solution and the plasma specimen. The correlation reduces the standard deviation very little because of the relatively large influence of the standard deviation of C_1 and of L_{e_2}.

value which is somewhat too high for concentrations below the concentration at which K centers and is somewhat too low at higher concentrations. The mean concentration about which this variability occurs is also shown in Table 7.

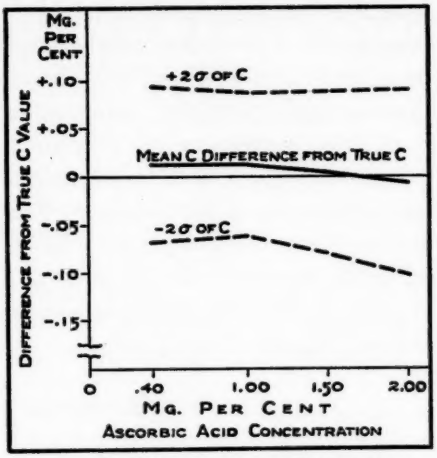

Fig. 2. Variation of ascorbic acid values for plasmas of four different concentrations to be expected from all errors of measurement about the mean difference from the true concentration when K is derived from solutions of 1.50 mg. and 2.00 mg. per cent.

The standard deviation of a plasma determination at a level of 1.5 mg. per 100 cc. is shown to be .040 mg. per 100 cc. and is 2.6 per cent of the determined amount, if one reading is taken on dye plus filtrate. As the K used centers at 1.75 mg. per cent, the mean value of a series of determinations is 1.504, and in about 95 per cent of determinations the derived values would be between 1.584 mg. per cent and 1.424 mg. per cent. This is shown in Figure 2.

The standard deviation of the ascorbic acid determination on plasma with a concentration as low as 0.40 mg. per cent is estimated as .040, and is the same as the standard deviation at 1.50 mg. per cent. Thus, although the percentage error increases steadily as the concentration decreases, the absolute error changes very little. If allowance is made for the mean overestimate at 0.40 mg. per cent, when K is at 1.75 mg., the probability is 95 in 100 that the determined value for a true value of 0.40 will be between 0.332 and 0.492 mg. per cent, and the mean of a series of determinations will be about 0.412 mg. per cent.

The errors estimated here for plasma determinations are, of course, those found for a single colorimeter and a specific group of laboratory workers. They do not represent the minimum error possible, but it is believed they are typical of what should be expected in routine work in a busy laboratory. The tendency for K to increase slightly at higher concentrations introduces a bias which is of little significance for diagnostic determinations, and the greater reliability of K derived from concentrations above 1.00 mg. per cent improves the accuracy of determinations. The major source of error in low plasma values is that associated with the accidental error in the measurement of dye reduction, and if greater accuracy is desired, independent readings should be made on two or three aliquots of the filtrate.

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ANNOTATIONS

NUTRITIONAL DEFICIENCIES[1]

DR. YOUMANS has written a book which should prove valuable to the medical practitioner desirous of obtaining practical information on the prevention, diagnosis, and treatment of nutritional deficiency diseases. He has adhered to a style "made familiar by the usual text-books of medicine." In most instances this has proven successful in providing a logical breakdown of the subject matter. The chapters on vitamins A, D, and ascorbic acid are particularly good.

A less strict adherence to a text-book style of writing might have increased the book's readability and practical value by decreasing its length through the avoidance of repetitious statements. The chapter on vitamin E, which is nine pages in length, might have been, advantageously, greatly condensed. The editing has not been carefully done. Too many sentences are complicated; others are difficult to decipher. For example, on page 92, we have: "In acute cases a toxic delirium characterizes the mental changes in the more chronic types of apathy, loss of memory, depression, and disorientation."

Much of the introductory chapter might easily have been dispensed with, devoted as it is to deprecation of multi-vitamin therapy, not readily reconciled with the author's belief in undoubted interrelationships among the vitamins (page 6); the importance and prevalence of those mild deficiency states which are so extremely difficult to diagnose (page 7); the characteristic multiple nature of nutritional deficiencies in humans (page 9); and his recommendation of "concentrates of food or food-like substances" (page 12). We have no argument with Dr. Youmans in his assertion that natural foodstuffs are to be preferred, provided that foods of sufficient quantity and quality can be obtained. However, we cannot agree that the use of combinations of vitamins is in general to be con-

[1] Youmans, John B.: NUTRITIONAL DEFICIENCIES. Philadelphia, J. B. Lippincott Company, 1941, 398 pp. $5.00.

demned. We believe that many occasions arise in the practice of medicine in which such preparations are useful and often provide the only means of insuring a more or less adequate supply of these substances for the patient.

We find it necessary also to take exception to the author's apparent interpretation of the terms "mild," "latent," "subclinical," and "hypovitaminosis," as synonymous. The item "hypovitaminosis" includes all degrees of vitamin deficiency below optimal nutrition and above complete avitaminosis, the latter being a most uncommon finding in man. "Mild" is a clinical term and includes all degrees of deficiency less than those which the clinician considers dangerous to the patient's health or life. The term "subclinical" signifies a state of suboptimal health which cannot be diagnosed by methods generally regarded as clinical. Conditions which are latent are asymptomatic.

The author uses the expression "safe minimum" daily intake. We believe that the only "safe minimum" is the optimum daily intake. Dr. Youmans probably would not agree as, for example, he recommends 3,000-4,000 I.U. of vitamin A as a safe minimum daily intake for adults, whereas the recommended daily allowance of the Food and Nutrition Committee, of the National Research Council, is 5,000 I.U.

The book contains an extremely interesting chapter on protein deficiency. Dr. Youmans makes two statements here which are quite provocative. Unfortunately, he fails to provide any very convincing evidence for their substantiation. The first of these is that relative deficiencies of protein are more frequent than deficiencies of the vitamins, and the second maintains that there is reason to believe that protein deficiency is one of the best single measures of general nutrition. A good measure of general nutrition is one which reveals early nutritional failure. Dr. Youmans has not shown that malnutrition in respect to protein (relative or absolute) can be detected reasonably early in the course of nutritional failure; in fact, the contrary appears to be true.

We believe Dr. Youmans' book to be a good one and that it fills a definite need. The criticisms which we have expressed are based on our belief that this book should have a wide distribution among general practitioners who might come to depend upon it as their Bible on nutritional deficiencies. Subsequent editions, therefore, should be, and undoubtedly will be, carefully edited and revised.

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